PRECLINICAL STUDY OF SIDDHA DRUG PAVALAVEERACHUNNAM FOR IT'S HEPATOPROTECTIVE, HYPOLIPIDEMIC,DIURETIC AND LITHOTRIPTIC ACTIVITIES

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DECLARATION BY THE CANDIDATE

I hereby declare that this dissertation entitled **"Pre clinical study of Siddha drug *Pavalaveera chunnam for its* Hepato protective, Hypolipidemic, Diuretic and Lithotriptic activities"** is a bonafide and genuine research work carried out by me under the guidance of **Dr.A.Kingsly M.D(S), Reader,** Post Graduate Department of *Gunapadam*, Govt. Siddha Medical College, Palayamkottai, Tirunelveli – 02 and the dissertation has not formed the basis for the award of any Degree, Diploma, Fellowship or other similar title.

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This is to certify that the dissertation entitled **"Pre clinical study of Siddha drug *Pavalaveera chunnam* for its Hepato protective, Hypolipidemic, Diuretic and Lithotriptic activities"** is submitted to the Tamilnadu Dr.M.G.R.Medical University, Chennai-32 in partial fulfillment of the requirements for the award of degree of M.D (Siddha) is the bonafide and genuine research work done by **Dr.J.Thilagavathi .**under my supervision and guidance and the dissertation has not formed the basis for the award of any Degree, Diploma, Associateship, Fellowship or other similar title.

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This is to certify that the dissertation entitled **"Pre clinical study of Siddha drug *Pavalaveera chunnam* for its Hepato protective,Hypolipidemic,Diuretic and Lithotriptic activities"** is a bonafide work done by **Dr.J.Thilagavathi,** a candidate of Government siddha medical college, palayamkottai, Tirunelveli – 02 in partial fulfillment of the University rules and regulations for award of M.D (siddha) - Gunapadam under my guidance and supervision during the academic year of 2016.

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Principal

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ABBREVATIONS

CPCSEA	-	Committee for the purpose of control and supervision of experimental animals.
DC	-	Differential Count
EDTA	-	Ethylene Diamine Tetra Aceticacid
ESR	-	Erythrocyte Sedimentation Rate
FTIR	-	Fourier transform infrared spectroscopy
Hb	-	Haemoglobin
IAEC	-	Institutional Animal Ethical Committee.
ICP-OES	-	Inductively coupled plasma optical emission spectrometry
ICP-MS	-	Inductively coupled plasma mass spectrometry
Ig E	-	Immunoglobulin E
LDH	-	Lactate Dehydrogenase
MCV	-	Mean Corpuscular Volume
PVC	-	Pavalaveera chunnam
OECD	-	Organisation for Economic Co-operation and Development
PCV	-	Packed Cell Volume.
PGE	-	Prostaglandin E
RBC	-	Red Blood Corpuscles
SEM	-	Scanning Electron microscope
TLC	-	Thin Layer Chromatography

CONTENTS

1. INTRODUCTION

Nature cures, not the physician

- Hippocratus

Natural Health is green health.

Siddhars were men of highly lectured intellectual and spiritual faculties combined with super natural powers. This system has been developed purely by the contribution of *siddhars* on their own line of thinking and achievements in the field & their research. This system of medicine was found by *siddhars* on the basic principles of nature and its elements, six taste, six seasons, *three Thodam* (*vada, pitha, kabham*) after careful and through study of human systems. This basic dictum in the science of life propounded by the great *siddhar*, Thirumoolar, three thousand years ago is as follows.

"Medicine means one that ensures physiotherapy
Medicine means one that ensures psychotherapy
Medicine means one that ensures prevention of disease
Medicine means one that ensures prevention against mortality

(Thirumoolar 3000)

The functioning of human body is governed by 96 specific faculties (or) *thathuvams.*

The word siddha comes from the word "Siddhi" which means an object to be attained or perfection or heavenly bliss. Siddhi refers to *Astama Siddhi*. These *siddhars* who attained or achieved the above said powers in life are known as. Accordingly "Man has five sense organs to perceive objects Thirukkural says,"

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In siddha system every drug is made up of five *boothams* and has got the following properties. They are *suvai* (Taste) *Gunam* (paroperty) *Veeriyam* (potency) *Privu* (Bio formto) *Mahimai* (effect)

Arusuvai is contributed by *panchaboothams:*

Inippu - *Appu + Piruthivi*

Pulippu - *Piruthivi + Theyu*

Karppu - *Vayu + Theyu*

Kaippu - *Vayu + Agayam*

Thuvarppu - *Vayu + Piruthivi*

Yuvarppu - *Theyu + Appu*

Tastes which provide by the *bootham* are classified as

Veppa veeria suvaigal - pulippu, Karppu and *uvarppu.*

Seetha Veeria Suvaigal- Inippu, Thuvarppu and *Kaippu*.

1. Catabolic potency - à w í i K ò ‹

2. Anabolic potency - Y î i K ò ‹

By knowing the cause of the disease we can change, the change the medicines by administrating the other type of *veeria* drug so that the affected *thosam* can be normalized.

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This line of treatment is called as '***Ethirurai***'

Sometimes drugs with the same *veeriyam* are also used to cure the disease and this sort of treatment is called "***Oppurai***" and mixed type of treatment is called "***Kalappurai.***"

Each and everything in the cosmos has its own inherent qualities and according to these properties it has got one to mole actions and effects called "***Gunam or porul panbu***".

Porul ponbu is a total action of drug. The same drug is administered to different diseases. But only the *Anubanam* is changed according to the particular nature of the disease.

Marunthu means which cure physical, Mental illness, which possesses preventive aspect from disease and also to postpone death.

The art of Practice of curing and treating illness and prevention illness is called ***Maruthuvam*** who practice such a science is called ***Maruthuvars.***

Pathiyam is a medical advice which includes life style modification and dietary modification as per diseased condition. ***Pathiyam*** which is peculiar to Siddha system of medicine.

Anubanam means it is adjuvant to medicine. It acts as a catalysts and enhances the rate of absorption of medicine.

They are many categories 25 varieties of water soluble(padanam – Arsenic), inorganic compounds (Karasaram - salt) 64 varieties, mineral drugs do not dissolve in water but emit, Vapor when put in fire.

It is a medical science through which the body as well as the soul are treated. It comprises of four main branches called *Vatham, Maruthuvam, Yogam* and *Gnanam*. The siddha system has not only the Gratitude and preventive effects on different diseases but also paves the way for longevity and immortality.

Siddhars used 5 form of Mercury.

1. Mercury - *Rasam.*
2. Red sulphide of Mercury - *Lingam.*
3. Mercury chloride (Terlinguaite*)* - *Veeram.*
4. Mercury subchloride (calomel) - *Pooram.*
5. Red oxide of Mercury - *Rasa chenduram.*

Research is necessary to solve such apparent middles of the transformation of these admittedly poisonous compounds. These inorganic group of drugs are usually

appreciated for their smaller dosage, long shelf life and easy palatability, most importantly for their sustainable quicker results in wide range of degenerative and refractory diseases. Present day siddha physicians also use many inorganic *chunnam* preparations in ailing human conditions like Rheumatoid arthritis,Cancer and HIV.

However there are a few higher order dosage forms like *Kattu, Karpam Kazhangu,Chunnam,* and *Gurukuligai* that are highly acclaimed in literature but seldom used by modern day physicians.

Siddhars methods of preparation of medicines are more scientific and involutes high order of chemistry science the global attention now turns towards alternative system of medicine and appreciated by the scientists and physicians belonging to different system of medicines. As the greatness of siddha system is now felt by every body.

My selection of the drug "*PAVALAVEERACHUNNAM*" is taken from "The Pharmacopoeia of Siddha Research Medicines" Authored by Shanmugavelu.M. In siddha system of medicine many formulations are available for the management of Liver disease, Jaundice (*Kamalai),* Gallstones, Billiarystones, Hepatic stones. *Chunnam* is a specialized medicine in siddha system. In current research an attempt is made to investigate, "*PAVALAVEERACHUNNAM*" for its Hepatoprotective, Hypolipidemic, Diuretic, Lithotriptic activities.

2. AIM AND OBJECTIVES

Aim

Scientific studies reveal that higher order medicines of Siddha like *Parpam ,Chenduram, Kattu, Pathangam ,Kalangu* and *Chunnam* contain nano particle. They have high therapeutic value in treating challenging diseases.The aim of this study is to do a scientific review to validate the safety and efficacy of '*PAVALAVEERACHUNNAM'* for Hepatoprotective by pre-clinical studies.

Objectives

The following methodology was adopted to evaluate the safety and efficacy of the test drug.

1. To collect the relevant Classical *Siddha* literature as well as Modern Sciences that supports the study.
2. To standardize the preparation of the drug according to classical *siddha* literature.
3. To subject the drug into Physico chemical analysis.
4. To analyze the drug chemically for the detection of acidic and basic radicals.
5. To Estimate the percentage of elements present in the drug by Instrumental analysis.
6. To evaluate the acute and sub-acute toxicity studies profile of *Pavalaveerachunnam* according to OECD guidelines.
7. To Establish the following Pharmacological activities:

- Hepatoprotective activity
- Hypolipidemic activity
- Diuretic activity
- Lithotriptic activity

3. REVIEW OF LITERATURE

3.1. *VEERAM (Savveeram)* :

3.1.1. GUNAPADAM ASPECT:

Synonyms :

Meenachimynthan, Kotchiveeram, Poovindhusevagan, Sarakku chunnam, Parangi Pasanam, Sarathin sathru, Parimithru

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According to the above version the synonyms are *Virpan, Aathi, Vellai chenduram, Sevagan, Pidaragan, Varagathinthalai*

Origin:

Veeram is one of the natural in 64 *padanam*s. Naturally it is obtained in Himalaya hills.

Now we use the artificial one. It is called as *Savveeram.* It is identified in seventh century and used for venereal diseases during the middle of the eighteenth century in western countries. Built for many centuries in siddha system the *Veeram* has been used in India for the treatment of various disorders.

Synthetic preparations (*Vaippu)*

Ingredients:

Calomel	- 80 parts
Culinary salt	- 80 parts
Copper sulphate	- 40 parts
Alum	- 20 parts

Potassium nitrate - 20 parts

Fuller's earth - 20 parts

Sulphate of iron - 10 parts

Ammonii chloridum - 5 parts

These ingredients are taken and triturated well and placed in a bottle its mouth is closed and sealed with a mud pasted cloth and burnt. After cooling, the per chloride of mercury is found to be de-posited over the lid as a thick layer.

Specialities :

- ❖ It is a very important drug for preparing *Parpam, Chendooram and Guru*
- ❖ It is having the capacity to make *kattu* of 64 drugs
- ❖ Make *karam into kattu* medicine form.

Methods of purification and detoxification:

Perchloride of mercury is as such quite toxic and it should be used only after purification and detoxification.

Perchloride of mercury 35gm is consolidified with pepper de-coction (Piper nigurm) for 6 hours. Then it is buried within the pep-per poultice. Sodium chloride 650 gm and camphor 35 gm are mixed well and kept in a mud pot in which the above poultice is buried and burnt for some hours with low intensity fire to get the purified form of perchloride of mercury.

Method I:

Camphor is mixed with tender coconut water and placed in a mud pot. Perchloride of mercury is tied in a cloth and soaked in the pot without touching the water and the pot is burnt for half an hour.

Method II:

Alum 35 gm and Camphor-35 gm. are powdered well and mixed together. Perchloride of mercury 35 gm is taken single piece and consolidified by the above mixture gradually. Perchloride of mer-cury is carefully watched not to become fume.

Method III :

Perchloride of mercury is soaked in mother's milk in a porce-lain vessel. It is isolated till the milk is completely dried to get the purified form. Cow's milk can be also used instead of mother's milk.

Method IV :

Bitter gourd (Momordica charantia) is opened and a hole is made, perchloride of mercury is placed in the center of the hole and closed, tied with a rope. Tender coconut water (or) lime juice is poured in a mud pot. The above tied material is suspended in the pot without touching the water and burnt for one hour.

Method V

Veeram is covered by pepper paste and tied in the cloth. Tender coconut water placed in the mud pot. The above material soaked in the pot without touching the water and the pot is burnt for one and half an hour.

Properties and actions of *Veeram*

Characters :

Taste	: Bitter and salty
Pontency *(veeriyam*)	: Hot (*Veppam)*
Pirivu	: Pungent

Bitter taste of *Veeram* has Earth + Fire elements.

It has got body improving tonic and antiseptic and ulcerogenic properties.

Panchabootha Amsam : Theyu

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According the *Veeram* is *Appubootham* in *pasanathil Panchapootham*.

In *Pasanapirivil Veeram* considered as *a theyu* (fire) *bootham.*

Veeram is classified as a *theyu sarakku* in *panchabootha sarakku* and also *the karasarakku* and *peesasarakku*.

The following five have been specifically mentioned as *Pancha boodha paadaanam* in the text "*Pacchai Vettu 16*".

1. Earth - *Prithivi* : *Arithaaram*
2. Water – *Appu* : ***Savveeram***
3. Fire – *Theyu* : *Gowri*
4. Air – *Vayu* : *Vellai*
5. Sky – *Vinn* : *Lingam*

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Ý ùªî£¼ êšió ñŠ¹ õ£°‹
ªðKî£ù ªè÷K«ò£ «î» õ£°‹
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However, in *Nandheesar's Kalaignanam the panchaboodha Paadaanas* have been mentioned in different way.

Fire – *Theyu* ***Veeram***

Substances Antagonist to *Veeram* (*Sathru sarakku)*

Iron(*Irumbu)*Magnet*(Kantham,)*Tablesalt*(kalluppu,)*Potassium nitrate(*Vediuppu)*, zinc *(Thuththanagam)* Egg white*(Muttai vellai karu*)

Substance Synergetic to Veeram

Copper(*thurusu*)

Actions:

Alternative *(udalthetri)*

Antibiotic *(Kiruminasini*)

Anti-septic (*Azhugal agatri*)

Caustic *(Punnundakki*)

General properties (*Pothu gunam*)

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Savveeram is used to cure the following disease

Gastric ulcer, leprosy, severe vadha diseases and morbid growth of flesh, throbbing pain associated diseases ,venereal diseases ,bubo in the groins occurs to the female and male due to forcefulness of sexually contact as explained in the above Tamil verse. This is also for various types of eye diseases

Dose:

2 - 4 mg

Dose above 4mg will be toxic.

Method of administration of *Veera Jayaneer:*

The perchloride of mercury and Ammonii chloridum (650 mg each) are taken and dissolved in 500 ml. of purified water and ad-ministered upto 30 drops.

MAHAVEERA MEZHUGU

Group-A

Perchloride of Mercury	35g
Drumstick tree bark juice	1.3 lt

Group-B

Calmel	35gm
Cinnabar	35 gm
Ceylon leadwort root bark (Plumbago zeylanica)	210gm
Dried ginger (Zingiber officinalis)	35gm
Black pepper (Piper nigrum)	35gm
Long Pepper(Piper longum)	35gm

Honey	Required quantity
Mother's milk	Required quantity

Group- C

Saffron (Crocus sativus)	4.2 gm
Bezoar orientale *(Korosanai)*	4.2 gm
Crude camphor *(Pachchai karpooram)*	4.2 gm

Perchloride of mercury is consolidified by the drumstick tree bark juice through heating and powdered. The 'B' group drugs are also powdered.

The A & B group powders are mixed well and triturated with the Ceylon lead wort root bark with the required quantity of honey and mother's milk. Finally group C drugs are added and triturated well until it becomes waxy consistency and stored.

Dose

Equivalent to the size of 1-2 pulse grain

Diseases cured

Vatha diseases and venereal diseases.

Dietary restriction

Milk and rice.

***VEERA MAATHIRAI (THIRI THODA MATHIRAI*)**

Purified *Veeram* (Perchloride of mercury)	1 Part
Pepper Seeds	4 Parts

Grind the both using pepper decoction and make the paste into pills of pepper size and dried. If given with suitable adjuvants the diseases like fever caused due to the derangement of three *gunmams* will be cured.

Instead of *milagu decction, Nochi Surasam*(Vitex negundo) is also used by which running nose and shivering fevers are cured.

***SAVVEERA CHENDOORAM* (Hydrargyri perchloride)**

Take equal weight of *Savveeram* and borax (*Vengaram*), pul-verize them and heat by putting it in a mud pan to get the *chendooram.*

If given in sufficient quantity it cures, fever, delirium, blabbery, severe *vatha* diseases, cholera, *soolai etc.*

SAVVEERA KATTU

Take 35 gms. of Camphor and put it in a mudpan (Agal) and put 35 gms of *Savveera* upon it. Again put 35 gms. of Camphor over it and put into *Lagu puda* process using mud cloth, and get the *Savveeram* of *Kattu* .

VEERA RASA PARPAM

Veeram (Perchloride of mercury)	10 parts
Cast Iron Powder	2 parts

Grind these two, by which rasam will come out, place the rasam in a mud pan and add salt. Then put into mud-cloth puda process by which we can get white fine powder, the *parpam*. Scrap the *parpam* and bottle up.

Dosage

488 mg or required minimum quantity with jaggery.

VEERA NEER (For Local application)

65 mg .of *Veeram* is mixed up with 240 ml. of water to get the *Veera Neer*. It is used as a disinfectant and for cleaning the ulcers.

VEERA KALIMPU

4.2 gm. of *Veeram* is ground with 17.5 gm.of butter and the paste is used to cure urticaria and to apply on the ulcers.

VEERA KALIMPU (AMIRTHA VENNAI-AMIRTHA MEZHUGU)

5.1 gm. of *Saweeram* is pulverized and ground with 350 gm. of butter for 6 hours, and cleaned with water.

It can be applied on die ulcers like Cancer or Carbuncle of chest

Clean the ulcers using the water boiled with tamarind leaves.

It is also used as local application on all swellings, boils etc.

It cures burning sensation in external piles.

As the *Amirtha mezhugu* contains more quantity of butter than *Veera Kalimbu* it cures the inflammation.

JAYAVEERA RANA SINGI KAYIRU

Boiled oil	42gm
Vengaram(borax)	42 gm.
Veeram((Perchloride of mercury)	4.2 gm.
Black gram flour	8.4 gm

Grind these in a *Kalvam* adding rain water and make pills in wax stage and dry them in shade. The colour may change from white into brown and which is harmless. It should be used after one year.

Adjuvant Pure water

Usage

Ulcers, ringworm, pimples, syphilis, scabies, carbuncles, cancer of penis, piles,scrofula,veneral ulcer,anorectal syphilis and different kinds of eye diseases.

OTHER PREPARATIONS

1 *Veera sanjeevi mathirai*:

Dose :*Ulunthu alavu* (65gms)

Indication :*Sanny, Suram.* Page 33

2 Maha koda surimathirai:

Dose :65gms

Indications: *vettai, sanny ,visa noi.* Page 38

3 Veeraparpam patchai vettu:

Dose : 50mgs- 3days- 6 times

Indications : *Pun,vellai,kiranthi,katti,soolai .* Page 46

4 Ramabana chenduram:

Dose : Arisi edai

Indications : *Vayu,Veekam,Suram, Megam.* Page49

5 *Thanvanthri sandamarutha chenduram:*

Dose : *Pana edai* -(480 mg)

Adjuvant : Honey

Indications : *Sunny 13 ,kuttam,**Mahotharam,Peeligai**,Gunmam,*

Vaginal cancer. Page 50

6 *Vadha marutha melugu*

Dose : *Milagalavu*

Adjuvant : Jaggery

Indications: *Mudakku ,Vadham ,soolai,Pandu,* Epilepsy,*Parisavayu.*

Page 54

7 *Candamarutha cenduram*:

Dose : Panavedei(480 mgs)

Adjuvant: Honey.

Indication *13 Sunny,**Peeli,** , vadham,* Fistula, *perumpadu*

Anuboga vaithiya bramha ragasiyam

8 *Veera paspam* :

Dose : *Panavedei*

Adjuvant : Honey

Indications : *Sunny, Thosam, kabakatti,vayu.* Page.505

Athmaratchamirtham

Uses of *Veeram*

1 ***Veera jayaneer*** Dose 30 drops

2 ***Mahaveera mazhugu*** Dose 65 - 130 mgs

3 ***Veera mathirai (Thurthada mathirai*)**

Dose : *Milagalavu*

Adjuvant : *Notchi* decoction

Indications : running nose, *kulir suram,*Fever

Saveera chendooram

Dose : 50-100 mgs

Indication *:* *suram, sanny, pithavanthi, pedigai, soolai*

Gurupathangam

Dose : 1/2 *Arisiedai* (30mgs)

Adjuvant : Palm jaggery, Dry ginger paste

Indications *:* *mehvayu,* various types of ulcers, check cancer, *soolai noi*

Navalogamelughu

Dose : 1/2 -1 *kundri* (65-130mgs)

Adjuvant : Palmjaggery, Dry ginger, Butter

Indications : Fever, *Chunny, Mehanoi, Giranthi pun*

Pattu karuppu

Dose : 2-4 *Arisiedai* (130-260mgs)

Indications : *Suram, mehasuram,* oedema, epilogsy

Anupagavaithiya navaneetham

External use of *veeram*

Veera neer 65 mg of *veeram* is mixed up with 240 ltr of water

Veera kalimbu Apply on the ulcers

Veera pugai 3 days and 6 times

Indication *Giranthipum,Araiyapu* non curable ulcers

Pilavaikku pasai Apply on the carbuncles

veeram cures all types of the skin disease by the external applications

Anubava vaithiyam Dr Thirumalai nadarajan

Veera pugai Cures the oozing ulcers

Veera powder Cures the chronic ulcers

Venkarapodi

18 kuttam, thimir, snake bite, dog bite, ulcers, piles, syphilis

Manjal karaseelai

Piles ,*marpani, neerabeelai ,Thimir, karanai katti*

Punpuraikku pugai

Dose 4.1 gms

Smoke curves *soolai* and ulcers

Moolam pavathiram punnukku pugai

Dose 4.1gms

smokes cures piles, fistula, ulcers.

Tholaiyu mennar sirodhararo **(ª î £ ¬ ô » ª ñ ¡ ø£˜ C ˆ î ó «ó£)**

Kustarogam, soolai, kai kal mudakku, pun, vadha karappan

Akkinipilasthiri

Ulcer curves all types of ulcers.

Agasthiar Nayana Vaithiyam

signs and symptoms

The toxic symptoms of mercury choloride are Taste of verdigris, Ulcerative stomatitis, Ulcerative gastritis, Vomiting, Watery diarrhea, Puffiness of face, Fissures on the skin with serous fluid oozing, Morbid thirst, Hiccough, Syncope, Ptyalism, Ulcerative laryngitis Dysphagia, Ulcers in the stomach, Dysentery, Pharyngitis, Throbbing pain in the hypochondral region, Unconsciousness, Convulsions, Death

Antidotes:

1. 20 ml. of Tribulus terrestris juice should be given to the victim in the morning and evening daily.
2. Paste of the Indigofera tinctoria root bark in the dose of a size of Solanum pubescens, dissolved in 80 ml. of lukewarm water should be consumed twice daily. Decoction of the above drug is also given as an anti-dote for the poison.
3. 20 ml. of juice of Vemonia cinerea consumed twice daily which also acts as an antidote.
4. This poison can be neutralized by drinking coconut toddy. Since the period of treatment is not mentioned, all the above prescriptions can be continued til the toxic effects disappear.
5. White yolk of the egg (unboiled) mixed with water or milk should be given often.
6. Tender coconut water also neutralises the toxicity of Mercuric chloride.

VEERAM

3.1.2 GEOCHEMICAL ASPECT

Terlinguaite is the naturally occurring mineral with formula Hg_2clo. It is formed by the weathering of other mercury -cotaining minerals. Discovered in 1900 in the Terlingua district of Brewsher country. The colour is yellow, greenish yellow brown or olive green.

Chemical name

Terlinguaite name in other language:

German	Terlinguait
Spanish	Terlinguaita
Tamil	*veeram*
Hindi ‘	Sowweera pasaram
Sanskrit	Sowweera

General

Chemical formula	Hg_2clo, (Hg_2^{2+}) Hg_2^{2+} cl_2O_2
Category	Mineral
Hardness	2.3
Strunz classification	3.DD.20
Crystal system	Monoclinic
Unit cell	a = 19.51 A^o
	b= 5.91 A^o
	c = 9.47 A^o
	β = 143.81^o
	z = 4
Ratio	a:b:c
	3.301:1:1.602
Unit Cell Value	V 644.75 A^o3 (Calculated from unit cell)

Identification

Colour	Sulfur- yellow,greenish yellow, brown
Crystal habit	Aggregates of equate to be elongated crystals,powdery,massive
Crystal	Monoclinic - prismatic
Symmetry	H-M symbol (2/m)
	Space group : c2/c
Cleavage	Perfect on [io1]
Tenacity	Brittle
Mohsscale	2.5
Hardness	2-3
Luster	Brilliant adamantine
Streak	Lemon-yellow, turning olive - green
Diaphanelty	Transparent to translucent
Special properties	9.22
Optical properties	Biaxial (-)
Refractive index	2.350 =2.640 = 2.660
Birefringence	δ = 0.310

Pleochroism weak green and yellow 2v angle measured:20^{o}

Alters to - turns olive - green on exposure to light

Classification of Terlinguaite

IMA Status : valid - first described prior to 1959 (pre - IMA) - “Grand fathered”.

: 3/D.06-10

- Nikel - Strunz 10^{th} (pending)ed

3:DD.20

3:HALIDES

D: Oxyhalides,hydroxyhalides and related double halides

D: with H_g

- Dana 8thed : 10.4.2.1

10: OXTHALIDES AND HYDROXYHALIDES

4: A_2 (O,OH) Xq *- Hey's CIM ref : 8.5.3*

8: Halides - fluorides, Chlorides , Brorrides and lodides also fluoborates and fluosillicates

5: Halides of Zn and Hg

Types occurance of Terlinguaite

Type locality:

Terlinguae district, Brewster co, Texus - USA Year of discovery 1900

Associated minerals at type locality

Montroydite Mecury,Kleinite Eglestonite Cinnabar,Calomel

Physical properties of Terlinguaite

Diaphaneity (Transparency) Transparent Transclucent

Lustre Adamantine

Colour: Yellow, yellow-green, brown (darkness to olive green on exposure to light), pale olive green in transmitted light

Streak Lemon yellow, light yellow

Hardness (Mohs) 2-3 Gypsum - calcite

Tenacity Brittle - Generally displayed by glasses and most non-metalic minerals

Cleavage Perfect on { - 101}

Density 8.725g/cm^3 (measured) 8.73g/cm^3 (calculated)

Habit

Striated Parallel lines on crystal surface or cleavage face

Prismatic crystals shaped like slender prims (eg. tourmaline)

Magnetism Non magnetic

Luminescence Non - fluorescent

General Terlinguaite information

Chemical formula	$Hg^{+}Hg^{++}Clo$
Molecular weight	452.63 gms
Empirical forumula	HgHgClo
Environment	oxidized portions of mercury deposits
IMA Status	valid species (pre - IMA)1900
‘Name origin	Named after its locality

Morphology

Crystal prismatic [010] and often somewith flattened on {001} rarely prismatic in a pyramidal direction also equal dimensional or thick tabular {001} pulverulent; massive aggregates of imperfect crystals (Dana 7^{th}ed 53)

Other information (Confirmation of Veeram)

- Turns black immediately when treated with hydrogen sulfide
- Ammonia causes a slower change to black
- Decomposed with separation of calomel by acetic hydrochloric and nitric acids

Optical properties:

	Semitransparent
Colour	lemon-yellow, canary-yellow, sulfur-yellow, becoming light olive-green on long exposure to light, amber - orange to nearly black rarely zoned.
Streak	very pale yellow
Luster	Adamantine
Optical class	Isotropic may be weakly
Occurrence	Araresecondarymineralformedatlowtemperature in hydrothermal mercury deposits.
Association	Calcite cinnabar, metacinnabar, mecury, eglostonite, kleinite terlinguaite, monotrydite, calomel, gypsum.

Distrubution

In the USA from Terlingura,Brewsher co Texas in the T.S clack Quicksliver mine about 37 km northeast of lovelock fitting district mineral co and the MC Dermitmine,Humbold co,Nevada from near the clear creak mercury mine New Idria district San Benita co California.InMexio at Huahuaxtla,Guerrero and EI Doktor, Queretaro.

(*veeram*) Hg_2 cl (or) HgclHg

TERLINGUIATE

Terlinguiate has been found with calcite, monotroydite and mercury and rarely with eglestonite and calomel. The crystallized variety is with one exception, found only on the calcite layer ,while the powdery form occurs on and in the pink matrix. The exception referred to shows terlinguaite (with a little calomel) directly on the pink matrix. Apparently there is no calcite present. The terlinguaite has not been found in closed in any other mineral except that it is embedded, together with montroydite in calcite layer. The crystals of terlinguaite sometimes in close mercury.

Crystal traces are resembled to monoclinic. In small striated prismatic crystals elongated parallel to the b-axis many forms observed.

Cleavage	perfect - (101)
Hardness	2 to 3
sp. Gravity	8.7
Luster	Adamentine
Colour	Sulphur - yellow changing to olive - green on exposure.prism crystals
Optically	α – 2.35
	β - 2.64
	γ -2.66

Strong disperstion , P<μ

Hg - deposits with calomel, cinnabar, eglastorite, kleinite

Optical mineralogy:

	Thin seat
Isometric	H 2
Spy	8.33 - 8.56
Colour	Pale yellow, Yellow brown
Handfree	Yellow orange, yellow brown oxiolizezone Hgde....
Crystal faces	Prism crystals, massive and powder

Savveeram similar to *veeram* pleochoic white to olive green colour.

[*Ref*:DANA II 52.56]

3.1.3 LATERAL RESEARCH ON VEERAM

Antibacterial Screening of *Kodasuri Veeravaippu*, A Siddha Salt Preparation

Accepted on: 06-03-2013; **Finalized on:** 30-04-2013.

Author : M. Sathish Kumar[1], M.R.K.Rao1, A.Ganesan[2], G.Rengasundari[3]

ABSTRACT

In siddha medicinal practice the use of plant extracts as well as inorganic natural preparations has a vital role as medicines. *Kodasuri veeravaiuppu* is one such formulation prepared by many inorganic compounds available in nature like Mercuric Chloride, Mercury, Sodium Chloride, Rock Salt, Potassium alum, Ammonium Chloride, Oxides of Calcium and Potassium, Copper Sulphate and Potassium Nitrate. It is proven medicine for rheumatoid arthritis and as an antibacterial. The present study was to test the antibacterial property of this drug on human pathogenic bacteria *Bacillus cereus, Bacillus subtilis, Proteous mirabilis, Citrobacter spp., Staphyllococcus aureus. Escheritia coli, Vibrio chlorae, Salmonella typhi, Pseudomonas aeuriginosa and Klebsialla pneumoniae.* The results were encouraging when compared to standard drug Ciprofloxacin.

PREPARATION AND STANDIZATION STUDIES ON VEERA MEZHUGU-A SIDDHA ANTICANCER FORMULATION:

International Journal Of Pharmacy and Pharmaceutial Sciences

ISSN-0975-1491 Vol 6,Suppl 1,2014

Authors: P.RAJALAKSHMI, C.SAVARIRAJ SAGAYAM, P.BRINTHA

ABSTRACT

Veera mezhugu is a siddha drug formulation which is often prescribed in cancer Theraphy.It is a poly herbo-metalic preparation comprising Veeram(Corrosive sublimate) Rasam(Mercury)Pooram(Calomel)Lingam(Cinnabar)Sudam(Camphor) Sambirani(Benzoin) Perungayam(Asafoetida) Vediuppu(Potassium nitrate) Navacharam (Ammonium chloride)Vengaram(Borax) Nervalam seed(Croton tiglium) and honey.In the present work this anticancer Siddha formulation is studied from process and product standardization point of view.Physicochemical parameters are determined for the end product as per Siddha Pharmacopoeia.Such kind of standardization studies will contribute in establishing scientifically the merits of Siddha herbo-metalic preparations.

3.2 CORAL

3.2.1 GUNAPADAM ASPECT

Zoological Name : Corallium rubrum (Linn 1758)

Synonyms:

Vithurumam,Thukir, Thuppu,Piravalam, Varithithandu, Senthandu maalai.

“æ˜ ,«è£¬ô êƒèªñ£O˜ ðõ÷‹ ªð‡ º^¶

c˜Šð´ ºŠH«ù£ ¬ì‰¶”

õ£¼Fî‡´ õ÷˜‰î ªê‰î‡´ î£¡

«ï¼¶, ªè£®«ò£¡ «ï˜Hóõ£÷ ñ£ø

É¼¼ «õ¬÷ ê£^Fò ªõ‡ñ£¬ô

ð£¼F ï£ñ‹ ðõ÷^F¡ «ð¼«ñ

-Sattamuni Nigandu -1200

¶Šðó^î‹ Hóõ£÷‹ ¶A«ó & MŸðõ÷‹

M^¶¼ñ‹ ¶õó‹

Namathepa Nigandu

“ðõ÷^F¡ «ð˜î¬ù«ò ðèó,«èÀ

ð£ƒè£ù ¶A˜¶Š¹ M^¶¼ðñ£°‹

¶õ÷^F¡ õ£¼F,°œ î‡´ñ£°‹

²òñ£ù ªê‰î‡´ ªè£®»ñ£°‹

ðõ÷^F¡ MŸð¡ù, °÷ñ˜^î è¡Q

Üöè£è ê£ŸP«î£˜ ñ£¬ôò£°‹

°õ÷^F¡ Hóõ£÷‹ MŸðõ÷ñ£°‹

ÃPò«î£˜ ° íªñ™ô£ƒ Ã˜‰¶ð£«ó”

-Bhogar nigandu 1200,

Ochi, Maamukku, Arasiya Antham, Uchithamadhu, Kamam, Thumbu, Lingam, Vadam, Vangathusam, Vakkanam, Pathikadagam

Above line says the coral is one of the marine products. It is one of the nine gems and *kadalpadu* thiraviyangal.

As per Hindupurana, the muscles of *velan* fell down in the sea and become *pavalam* during his battle with lord Indiran.-

The corals are the exo- skeleton of the minute marine organisms. The skeleton of colories which are branched in all directions resemble tiny trees. These corals are found in the deep seas like Indian Ocean and Mediterraneamsea.

Thiruvilayadal puranam explained how the Coral should be identified by appearance. There are six good characters and six bad characters seen in *pavalam* depends on its appearance.

Origin:

Malthevs, Laxathevs, Rameshwaram in India. Coral also get from Japan, Subathra islands.

In Sambasivam Pillai *Agarathi* says, it is a band calcareous substance found in the Ocean, secreted by marine polys for their common support and habitation. The stony skeleton secreted by them continuously grows like plants which are called coral reefs.

Speciality of Coral:

Thirumoolar compared *Pavalam* with "Sakthi" (Lord Parvathy i.e. energy) and sulphur (*shakthi beesam*)

He mentioned in his quotes as follows,

«îM »óˆ¬î«ï˜ ªêŠðô£°‰ ¶Š¬ð

ò£M Jóî«ï ó£° ñ¾ˆFè‰

«îM òó¡ dê… ªêŠ¹ I¬õè«÷

«îõ˜ ºîô£«ù£˜ «î˜õ K¬õè«÷.

As mentioned in Silapathigarm, the good coral should not be twisted porous and must process bright red colour.

Purification of *pavalam:*

1. Coral (35gms) is socked in 210gm of dates, toddy and insolated. Fresh dates toddy is added on the next day and dried in sun light. This process is repeated for five times and the material is washed in water to get it purified.

2.Coral is boiled in cow milk for 3 hours (oru saamam)

- *Anubava Vaidhiya Kalanchiyam*- page-286

3.Coralreef is boiled in curd for 3 hours.

- *Pallandu vazha payanulla Marunthugal* - page 107

4.Coral reef is packed with a cloth and soaked in buttermilk and heated for one hour and thirty minutes (*4 Nazhigai*)

- *Yagopu vaidhiyam 300*- page 16

Character:

There are six good properties and six defects in *pavalam*.

Good characters :

Chinduram, Semmani, Senkai, Muchumuchukkaikani, Veeraikkani, Thuthulai Kani.

Bad characters:

Pilavu, mudakku, Thirukal, Thulai, Karuppu, Veliral.

In Silappathikaram,

è¼Šðˆ ¶¬÷ò¾ƒ è™L¬ì º ìƒè½‹

F¼,° cƒAò ªêƒªè£® õ™L»‹.

General properties *(pothugunam*)

Coral gives shiny appearance to the body. It reduces phlegm, cough and loss of appetite which occurs during the fever. It also counteracts the poisonous effect of insects. It also controls thirst and improves spermato-genesis.

èó«î£ì‹ äò ºˆ «î£ì²óƒ è£ê‹

Ü¼CW ìˆîô£‹ Ýô‹ & ªð¼Mˆ¶

ï†ì‹ ÜFî£è‹ ï£õø†C «ð£ªñ£O»ƒ

A†´‹ ðõöˆî£Ÿ «èœ

Coral has also got diuretic, laxative and astringent properties. It also strengthens the nerves. Further it reduces excessive phlegm.

The coral is useful in the treatment of *pitha*, excessive phlegm and eye disorders.

-Materia medica mineral animal kingdom.

Actions:

Expectorant	- *(Kabakari*)
Nervine tonic	- (*Naramburamakki*)
Astringent	- *(Thuvarpi*)
Diuretic	- *(Siruneer perukki*)
Laxative	- *(Malamilakki*)

Materia medica- (Mineral animal kingdom)

Emetic

Anti-bilious

Anti-Phlegmonous

- Indian materia medica vol.II page-157.

In *Pathartha soodamani*,

¹¬óJô£Š ðõ÷ˆî¡¬ñ ¹è½«õ¡ ÞQ¶«è‡ñ£
²ó«ñ£´ õø†C Í„²ˆªî£Œõ¼‰ î£è‹«êì‹
Þ¼ñ«ô õ£îHˆî I¬íðò¬õ c,°ªñ¡Á
õóñLºQõ˜ Cˆî˜õ°ˆî õ£èì*f*èœ ªê£™½‹

- Agathiyar vagadam

¶A¬ó«ò Ü¿,ªè£N‰¶ ¶Mîº‹ð‡ í£ñ™
õA˜õ¼õ¬ù òõ¡è‡ õQ¬î«ò ÞQ¶«è‡ñ£
àA˜ºQ Þóˆîè£ê‹ àŠðê‹**õ™¬ô** «ê£¬ð
ïM˜õî£ ªêŒc˜ H¡ù˜ïM™ªõù ªõ¡Áº¡«ù.

Coral reduce thirst, tasteless, tuberculosis, **jaundice**, fever, anemia, Poisonous effect of insects, spermatogenesis. *- Gunapadam kaiedu*

Dose :

3 - 12 grains - (200mg to 800mgs)

Thrice a day after meals. - *Indian materia medica - page 157.*

Preparation of *Pavala parpam*

1. The coral is triturated with latex of *Erukku* (Calotropis gigantea) and kept in an *aghal* and put to *puda* process to obtain the *parpam.*
2. Poultice of *Ilandai* (Zizyphus jujuba) or *thaivelai* (Gynandropsis pentaphylla) is applied over the coral and kept in an *aghal* and put to *puda* process. It is then cleaned with cow's milk, insolate, powdered and taken.
3. The parpam can be prepared by using *keezhanelli* (Phyllanthus niruri).
4. The Coral could be triturated using rabbit's blood and made into cakes and put to *puda* process.
5. Equal quantity of coral and *karpoora silajat* triturated using juice of the *vqjravalli* and put to *gaja puda.* The process is repeated with juice of Oxalis corniculata *(puliyarai juice)*
6. Coral(20gm) and honey (120gm) are placed in **a** new mud pot. The pot is tightly covered with a tile. A mud pasted cloth is made put and to *pudam* to get the *parpam.*

Pavala Chendooram:

Coral (35gm) is triturated individually with the following juices of palmyra, cotton (Gossypium herbaceum), Viola suffruticosa, Daemia extensa, Arecanut and wild chinchona.

It is then subjected to puda to ob-tain *chendooram.*

This *chendooram* is effective in the management of piles, burning sensation of head and hand, abdominal pain,tremors and diarrhoea

Pavala Chendooram:

Adjuvants	***Indications***
1. Hot water	Bleeding haemorrhoids
2. Sugar	Fistula
3. Ghee	Burning sensation of head and hand
4. Juice of Allium cepa	Peptic ulcer with vomiting
5. Panagam	Tremors
6. Water	Morbid thirst
7. Liquorice	Soothaka Sanni
(Glycyrrhiza glabra)- *A dhimaduram*	
8. Cajanus cajan leaf juicefPigeon pea)	Abdominal pain
9. Crataeva religiosa	Diarrhoea

Adjuvant : Honey,Ghee.

Indications: Piles, burning sensation of head and hand, abdominal pain, tremors and diarrhoea.

Other preparations

Pavalaparpam

Dose : -3 *Kuntri* (65 – 400mgs)

Adjuvant : Ghee, Honey

Indications : *Kannoi*, *Uppusam*, Vamiting, ***Vallaikatti***, Asicitis,

Pavala parpam

Dose : to 3, *Kundri*(65-400mgs)

Adjuvant : Coriander leaf juice

Indications : Dysentery, *Pitha Noi*.

Pavala parpam

Dose : 1-4 *Kundri* (130 - 520mgs)

Adjuvant : Butter,

Indications : *Manneeral veekkam* (splenomegaly) ,diarrhoea

Pavalaparpam

Dose : 2 -4 *kundri* (260-900mgs)

Adjuvant :Butter, Ghee

Indications : Cough, TB, ***Pithanoi***, *Vettai*

-Anuboga vaithiyanavaneetham (Hakkim) 3rd part

Karuvanga chenduram

Dose : 2 - 4 Kundri (260-900mgs)

Adjuvant : Suitable one

Indications : *Vettai Noi, Meha Noi*

Anubopa vaidhiyam - page - 83

Thirilokkia chinthamani

Dose :1 *varagan* (3.5gms)

Adjuvant : Honey, Pepper, Ginger juice, Dryginger water

Indications : ***Kavisai***, Soolaikattu, Anemia, Abdominal Pain, Asicits, Fistula. Epilepsy

-Eliya vaidhiya muraikal (S. Prema) - page 248

Pavalaputru Parpam

Dose :2-4 *kundri*(260-900mgs)

Adjuvant : *Elanthiver, Elanthai palam*

Indications : Menorrhegea

-Anboga vaithiya murai Dr. C.N. Kuppusamy -page 40

Rajamirugangam

Dose : 520mgs

Adjuvant : Pepper, Honey

Indications : Reduce body heat

Vasantha Kusumaharam

Dose : *ulanthu alavu* (65 mg)

Adjuvant : Honey, Jaggery, Ghee

Indications : Anemia, Dysentry, *Soolai*, Piles, **M*eharogam***, Chest pain, TB,

Page – 99

Eraniyakarppam Rasam

Dose *: Ulunthalavu* (65mgs)

Adjuvant : Suitable one

Indications : *Vadha, Pitha, Kaba and Thontha suram* Page – 120

External uses

Thumalaravu vellaipravalathi Mathirai

Adjuvant : *Nanthiyarathi* juice

Indications : Eye disease (*Nethrarogam)* Page - 67

Sethupandithar Rathinathi mathirai

Adjuvant : Breast milk,

Indications :Eye diseases

Rathinathi mathirai

Adjuvant :Brest milk (or) Palm leaves juice

Dose : *Venthayam Alavu.*

Indication : All eye diseases - *Thimiram, Kasam, Padalam, Sukkan, Ranasukkiran, Pillam*

CORAL

3.2.2. ZOOLOGICAL ASPECTS

Classification

Kingdom	- Animals
Sub-kingdom	- Radiates
Infra-kingdom	- Coelenterata
Phylam	- Cridaria
Class	- Anthozoa
Sub-class	- Alcynoaria (octocoralia)
Order	- Gorgoncea
Family	- Corallidae
Genus	- Corallium
Species	- rubrum

ZOOLOGICAL NAME

Corallium rubrum (Linn 1758)

SYONYMS

Madrepora rubra (Linn 1758)

Isis nobilis

Gorgonia nobilis (Linn 1789)

www. sn2000 taxonomy.nl/main/classified

Meaning of Coral

 A rockline deposit consisting of the calcareous skeletons secreted by various marine inverstebrates.Chiefly anthozoans,coral deposits often accurate to form reefs or island in warm sees.

 A polyp or colony of polyps of any of the numerous anthozoans that secrete a hard or flexible skeleton espacially the reef building hard corals.

 A polyp or colony of polyps of any of various hydrozoans that secrete hard skeletons, such as the fire corals.

 The hard skeleton of various corals espacially of red corals of the genous corallism used to make Jewellary and ornaments.

In Zoological view.

Any marine mostly colonical coolenterate of the class on the zoo having calcareous horny or soft skeleton.

Coral are invertebrates like shallfish they have no spinal colounm or internal bones.Coral colories are composed of many tiny cup-shaped animals called polyps.Millions of polyps working together in a co-operative.Colony generation after generation create the limestone skeletons that form the framework of the beautiful reef.

Corals losely defined include stony corals otocorals.Black corals and hydro corals,constituting approximately 4820 species.65% of those species or about 3,150 occur in deep water.

There are two general types of corals

* Hard Corals
* Soft Corals

Hard Corals

Hard corals usually grow in large colonies and make up coral reefs.They create skeletons out of limestone. Which eventually becomes the rocky structure most of us are familiar with hard corals survive on tiny algae and sometime small fish like plankton when people talk about “coral” they are usually taking about hard corals.

Soft Coral

Soft corals often seem like plants or trees.These intestesting corals don’t have stone like skeletons they have woody cores and fleshy rinds for protection.They are non-reef building corals and don’t necessarily need algae to survive.They ‘re naturally found in a variety of climates both tropical and cooler regions.Many soft corals are very easy to keep and are suitable for a beginner tank.

Varieties of coral:

Many varieties of coals are like red corals,black corals,white corals,blue corals, soft corals,stony corals,horny corals,pepper corals and organ pipe corals

Types of corals:

1 Pillar coral:

Scientific Name: Dendrogyra Cylindricus

2 Stagborn coral

Scientific Name:Acropora cervincornis

3 Sea pen

Scientific Name: Pennatulacea

4 Coenthecalia (Blue coral)

Scientific Name: Heliopora coerulea

5 Elkborn coral:

Scientific Name: Acropora palmata

6 Leptosammia

Family: Dendrophylidae

7 Antipathes (Black coral) - 100 species

Scientific Name: Antipathes

8 Great star coral(stony coral)

Scientific Name: Monokastrace cabernosa

9 Colpophllia natans'

Scientific Name: Colpophyllia natans

10 Diploria strigos

Scientific Name: Diploria strigosa

11 Eusmila (Stony coral)

Family: Meandrinidae

Coral reefs:

Coral reefs are diverse underwater ecosystems held together by calcium carbonate structure secreted by corals.Coral reefs are built by colonies of tiny animals found in marine waters that contain few nutrients.Most coral reefs are built from stony corals,which in turn consist of polyps that cluster in groups.The polyps belong to a group of animals known as cridaria,which also includes sea anemones and jellyfish.Unlike sea

anemones,corals secrete hard carbonate ecoskeletons which support and protect the coral polyps most reef grow best in warm shallow, clear sunny and agitated waters.

Often called “Rainforest of the sea”shallow coral reef from some of the most diverse ecosystem on Earth.

Types of coral reefs

Most reef scientists generally recognize three major types of coral reefs

1 Fringing reefs

2 Barrier reefs

3 Atolls reefs

1. Fringing reefs

They are reefs that grow directly from a shore while there may be areas of shallow intertidal or sub-tidal sand bottom lying between the beach and the inshore edge of coral growth there is no lagoon between the reef and shore.

2 Barrier reefs

They are extensive linear reef complexes that parallel a shore and are separated from it by lagoon.

Barrier reefs are far less common than fringing reef or atolls although examples can be found in tropical Atlantic as well as the pacific.

The 1200 mile long great barrier reef of the NE coast of Australia the word largest example of this reef type.

3 Atolls

An atoll is a roughly circular(annular) oceanic reef system surrounding a large (and often deep) central lagoon.In the south pacific most atolls occur in mid ocean.

Atolls are usually circular or oval in shape,with an open lagoon in the center.

Other Types of reefs

Apron reef

Patch reef

Ribbon reef

Table reef

Platform reef

Coral reef can take a variety of forms as the following

Apron reef

Short reef resembling a fringing but more sloped,exhending out and downward from a point or penisular shore.

Patch reef

An isolated,often circular reef,usually within a lagoon or embayment.

Ribbon reef

Long narrow some what winding reef usually associated with an atoll logon.

Table reef

Isolated reef approaching an atoll type but without a lagoon.

Platform reef

Usually lie in sheltered seas and quite for affshore.They are flat-topped with small and very shallow lagoon.

Formation of coral reef

The animal nature of coral reef was established in 1927.Present reef budding corals donot grow below 50 meters.Geological evidence indicates that the coral polyps of the past ages were also littoral in their habits.Several theories were propound in the past to explain the mystery of origin of coral reef and atolls of which the following are the chief ones.

1 South buryra valcanic crater theory

2 Darwin's Dana substance theory

3 Semper's Murray solution theory

4 Doly clacial control theory

The idea of an aribecatant platform of foundation is necessary postulation for all coral growth.As there are many kinds of such foundations and many many geological phenomena forming them,it is natural that no single thorey can account for all the cases reef formation.

Age of the coral reefs

Most reefs grew at the rate of 10-200mm each year.Most of the existing reef could have been formed within a period of 15000 to 30000 years.

Composition of coals

	Cao	**Mgo**	**Sio2**	**Loss on ignitiam**
Hydroza Alcicorris	52	0.43	0.23	44.77
Actinozoa poritec Astreoides	53.84	0.18	0.02	44.98
Madrepora prolifera	53.48	0.06	0.19	44.50
Favia fragum	53.69	0.18	0.28	44.44
Maeandral abyrinthiformis	53.71	0.26	-	45.10

Physical properties

Density	2.60 and 2.70 gml cm^3 (usually)
Refractive indices	nz 1.49
	nv 1.65
Hardness	2.5 j 4 according to mohsm scale

Constituents

Animal (or) organic matter	8%
Carbonate of lime	83%
Magnesium carbonate	3.5%
Oxide of iron	4.5%

The red colour is due to its containing iron.

Ref : Indian meteria medica Dr KM Nadharri

Medical uses:

Pseudopderosin found in a caribbean gorgonian coral. This chemical is used in a cream that protects the skin from weather damage .It also is being investigated as an anti inflammatory for we in conditions such as prosiasis and contact dermatitis.

3.2.3. LATERAL RESEARCH ON CORAL

ANTIBACTERIAL AD HAEMOSTATIC ACTIVITIES OF A SIDDHA FORMULATIO – *PAVALA PARPAM*

Pharmacologyonline* 1: 613-624 (2011) Thanigavelan *et al.

Author Thanigavelan.V,Victor Rajamanickam.G.Kaliyamurthi.V,Lakshmanakumar.V, Sasikala.N andThirunavukkarasu.S.V

Background: *Pavala Parpam* (PP) is a traditional Siddha medicinal preparation. This marine sourced medicine is synthesized through calcination of Corals as narrated in the classical Siddha literature – *Anuboga Vaidhya 'avaneetham*. This literature evident shows that *Pavala Parpam* has astringent action and becomes evident in arresting bleeding. The primary objective of this work was to validate the safety and haemostatic efficacy of PP. *Methods:* The raw *Pavalam* were procured from country drug store at Marthandam, Tamilnadu and purified by the traditional procedure by soaking in lemon juice for 24 h and the test drug PP was prepared by the process of *Pudam* (Calcination) described in Anuboga Vaidhya Navaneetham 3rd part, page no: 132-133. Adrenochrome and other analytical grade chemicals were procured from Sigma chemicals, U.S.A and S.D fine chemicals Ltd, Mumbai. The experiments include preliminary biochemical studies by standard methods, quantitative analysis of Calcium by AAS, antibacterial studies by paper disc diffusion method, acute oral toxicity study under OECD 423 guidelines, and the haemostatic effects of PP in Albino mice including shortened bleeding and clotting time efficacy on by the method described in Ogle *et al*.,1977. *Results:* The qualitative and quantitative analyses of PP show that it has the contents of 37.48% of calcium, ferrous iron, tannin, and tannic acid. These compounds have the property of Haemostatic action. *In vitro* studies, *Pavala Parpam* has good anti microbial activity at the dilution of 25 microlitre/disc against the bacterial strains such as *S.mutans, S.aureus, E.coli, K.pneumoniae and P.aeruginosa*. Animals were found to be safe up to a maximum dose of 2000mg/kg body weight in acute toxicity studies. The experimental studies done on animal model, *Pavala Parpam* shows potent Haemostatic action by exhibiting significant reduction ($P<0.001$) in bleeding time and clotting time of blood compared with control group. *Conclusion: Pavala Parpam* is the safest and efficacious haemostatic drug comparable to Adrenochrome – a standard drug.

3.3 BILE

3.3.1 GUNAPADAM ASPECTS

The biles of animals such as cow, buffalo, goat, deer, pig, dog, cat, peacock, fish and snake are used as medicine.

Bile has got laxative property. It is used to triturate the ***vairava*** pills which are useful for treating the diseases like delirium. It is effective in the treatment of white leprosy and is used as follows:

Process :

Vernonia anthelmintica ***(kaattu cheeragam)*** and dried leaves of Fumaria parviflora ***(thara)*** are taken in equl quantities. ***Chooranam***is made and placed in a mortar. The goat's bile is then added and ground for four days and pills are made in the size of a strychnos potatorum ***(thetran)*** and dried in shadow. Pills are taken

Dose :

Twice daily for three months.

Uses :

It is also applied topically.

It is also useful in the purification of steel.

Dried root of Trianthema decandra ***(saththi chaaranai)*** is placed in goat's bile. The root is rubbed in the flower juice of Tabernaemontana divaricata ***(nanthiya vattam).*** When the juice is used as eye drops 96 types of eye diseases are cured.

Siddha Medicines prepared from bile

Ananda bairavam	Snake bile
Karavala vairavam	Dog bile
Karunya vairavam	Pig and peacock bile
Sannipatha vairavam	Buffalo,Peacock fish bile
Siddha vairavam	Fish bile
Boothavairavam	Cot bile

Madhanavairavam	Cow bile
Manokaravairavam	Donkey bile
Vidhana vairavam	Deer bile
Vijaya vairavam	Dog bile

-Siddha vaithiya thirattu

3.3 BILE

3.3.2 ZOOLOGICAL ASPECTS

Bile is a digestive juice that is secreted by the liver and stored in the gall bladder. It has two important functions.

- Assists with fat digestion and absorption in the gut.
- Is a means for the body to excrete waste products from the blood.

➢ Bile does not contain enzymes like other secretions from the gastrointestinal tract. Instead it has bile salts(acids) which can.

➢ Emulsify fats and break it down into small particles. This is a detergent like action of bile.

➢ Helps the body absorb the breakdown products of fat in the gut. Bile salts bind with lipids to form micelles. This is then absorbed through the intestinal mucosa.

➢ The other important function of bile is that it contains waste products from hemoglobin break down. This is known as bilirubin and is normally formed by the body as it gets rid of old red blood cells which are rich in hemoglobin .Bile also carries excess cholesterol out of the body and ‘dumps it into the gastrointestinal tract where it can be passed out with other waste matter.

Composition of Bile

The bile of man and carnivorous animals is of a deep orange red colour,turning of greenish brown by decomposition of its colouring matter. In herbivorous animals it has some shade of green when quite fresh.But turns to a muddy brown after a time. It is transparent and more or less viscid according to the length of time it has remained in the gall bladder. It has an alkaline reaction. Its specific gravity is about 1005 when taken from the bile ducts directly, but it may rise to 1030 after prolonged stay in the gallbladder owing to the addition of mucus and the absorption of time of its fluid.

Approximately the proportions of the chief constituents of bile:

Water	85.0 percent
Biesalts	10.0"
Colouring matter and mucus	3.0"
Facts	1.0"
Cholesterin	0.3"
Inorganic salts	0.7"
Total	100.0

Bile contains no structural elements nor any trace of aluminous bodies.

* Lecithin
* Sodium
* Potassium
* Calcium
* Chlorine
* Bicarbonate ions

As mentioned, gallbladder bile is concentrated compared to liver bile. Bile salts make up the largest volume of gallbladder bile and can be 6 times more concentrated than bile salts in liver bile.

Metabolic Functions

Emerging evidence associated FxR activation with alternations in triglyceride metabolism glucose metabolism and liver growth.

3.3.3 LATERAL RESEARCH ON BILE

BILIARY SECRETION IN SUCKLING GOATS: THE EFFECT OF MATERNAL MILK AND OF A LAMB MILK REPLACER

Archives of Physiology and Biochemistry 1381-3455/96/10402-0239

1996, Vol. 104, No. 2, pp. 239-245 © Swets & Zeitlinger

Author A. Rueda, A. Valverde, J.A. Fernandez, E. Martinez-Victoria, J.A. Naranjo and M. Mañas

ABSTRACT

A total of 45 preruminant goats were fed either goat milk or a milk substitute. The postnatal development of bile secretion and biliary lipids composition of suckling goats and the influence upon these parameters of maternal milk substitution were studied during the first month of life. Samples of hepatic and gallbladder bile were obtained from kids of 1, 7, 14, 21 and 28 day-old. Bile flow rate, cholesterol, total bile salts and phospholipids were measured.

Our results show that the quality of the protein and fat used in the elaboration of the milk replacer, affects the evolution of both hepatic bile flow and biliary lipids composition. Differences between gallbladder and hepatic bile composition show a scarce concentration capacity of the gallbladder with both diets and at all ages studied.

CHUNNAM

3.4 PHARMACEUTICALS REVIEW

Definition :

The word '*Chunnam*' indicative of an alkaline product, similar to lime. In Tamil language, Caustic lime is also turned as *Chunnam*. The word denotes which colour (chun) lime (*chunnam*) fine particle and calcined paste.

Raw meterials in chunnam preparation:

Many metallic and non metalic mineral drugs are made into chunnam preparations.

Metals : Gold *(Thangam*) Silver (*Velli*) Copper (*Chembu*) Iron (*logam*) Zinc (*Naagam*) lead *(karuvangam*).....

Mercurials and other toxic substance : Mercury (*Rasam*) Cinnabar (*lingam*) Calomel (*Pooram*) Mercuric perchorid (veeram) *thalagam* (As_2S_3).

Salts (karasaram): fuller's earth *(Pooneeru*) salt peter (*vediuppu*) Camphor (*Karpooram*) *Kalluppu* (salt).....

Mineral origin : (*Uparasam*) Mica (*Abrakam*) asbestos (*kalnar*) Copper Sulphate (*thurusu*) Chalcopyrite (*nimilai).....*

Zoological product : *Kizhinja*l (conch shell) *Sangu, nandu odu, nathai odu, Muttai odu.*

Equipments required :

: Mortar and pestle

: Vessels and spoons to handle liquids.

: Long ribbons of tough cloth and fine clay.

: Pairs of shallow earthen discs of identical dimensions.

: Cow dung cakes, sufficient numbers and well dried.

: Fine cloth pieces for filtering juices and decoction.

: Spatula for handling powders.

: Air tight containers.

General Method of preparations :

The above naturally obtained ingredients are processed sequentially by elaborate processes like grinding melting, triturating, quenching in different plant juices with different heat treatments thus changing form from one to another and in the end, making into bio-available form.

Chunnams are prepared by two different methods one is simple and another is complex. *Chunnam* should be prepared during the hot. Summer months like April and May .Simple, Complex Method, Crucibles (*Moosai*) used in *Chunnam.*

Simple Method

In this method either lime stone or calcium group of raw materials are subjected to calcinations or incineration in *pudam* method with cow dung cakes as specified in the formula. Commonly these medicines are used extensively by physicians; examples are *chunnam* of salt peter (*vediuppu chunnam*) and *chunnam* of egg shell (*anda odu chunnam*), which are used extensively in urinary, genital disorders and in acid peptic diseases.

Complex Method

A very systematic, elaborate, stepwise procedure is followed in these preparations. High metals, gems or any toxic material is detoxified as per recipe and then converted it to a fine homogenous substance by triturating with plant juices or some distillates called *pugai neer* or *thiravagam* or with any solvent (*jeyaneer*)and made into fine paste and are well dried. Tins paste is kept in distinct, specially made crucibles called *moosai* like *Pancha chunna gugai* and sealed. After drying, this crucible is heated in a hand bellows blower.After sufficient heating, generally up till a point when the crucible becomes red hot and itself gives-up its structure. The contents are allowed to cool and collected. The final product after sufficient grinding

Crucibles (*Moosai*) Used iu *Chunnam*

It is very interesting to note that heat resistant crucibles (*moosai/ gugai*) are specially made prior to *chunnam* preparation Special crucible called *pancha chunna gugai* is prepared

with *chunnam* of crab shell (*nandu*).fresh water shell *(nathai*). conch shell (*sangu*). pearl oyster shell (*chippi*). egg shell (*anda-oodu*). latex of *Calotropis erukkampaal*;. egg albumin *venkaram* and lime water (*chuunna neer)*. *Anju chunna gugai*..*pancha butha gugai* are few other crucibles particularly used for *chunnam* preparation.

Product Grades

The c*hunnam* of many metal and non metal drugs are graded according to the solvents used in the preparation, the type of crucibles used and type of heating appliances used in the process of particular preparation. If the *chunnam* is prepared in *pancha cnhuna gugai* and incinerated with bellows blower is graded as best quality. If the *chunnam* is prepared with fuller's earth- *(pooneeru chunnam)* the drug is graded as second quality. If c*hunnam* is prepared by the use of copper sulphate *chunnam* (*thuiusu gum chunnam*).it is classified as third quality. If *chunnam* is prepared with caustic solvents like *jayaneer*. This variety is classified into 4th quality. Generally physicians prepare a special kind of solvent called *chunna thiravagam* to prepare all kinds of *chunnam* of salts, minerals, metals and various gems.

Shelf Life

When properly stored, they retain then potency up to 500 years.

Adjuvant - *Anupanam*

It is advised to take the chunnam medicine with ghee, butter or milk. Dosage is indicated according to the disease condition as 3 days or 7days or 21days.

Colour :

*Chunnam*s are in powder form and generally soft white to ivory color or colourless.

If white color is not obtained fuller's earth *chunnam* is added to get the white colour .

Character and tests for chunnam:

Chunnams are odorless, lusterless, smokeless on heating.

Microfine in particle size wafer like very light in cut

The end point on purity of *chunnam* is ascertained by adding a pinch of turmeric with few drops of water it turns turmeric from yellow to red perhaps as on indicater of the ^{P}H value.

Chunnam also testes like lime and will produce irritation when it comes in to contact with mucous membrane. *Kadunkara Chunnam* is highly alkaline caustic and blisher forming.

Preservation and storage:

Chunnam should be kept in dry well stoppard glass bottles.

Uses of Chunnam

Generally chunnams are acclaimed as best possible medicaments in chronic degenerative human ailments with various adjuvants. Chunnam of salt peter, which is called as vediuppu chunnam, is used extensively by present day physicians in miliary disorders especially on urinary calculi, and in some types of malignant and non malignant tumors.

Chunnam of borax which is called as vengara chunnam are highly preferred in metabolic disorders of liver and spleen diseases. Many of these chunnam preparations play a vital role in the process of higher order medicines called Kattu (solidified metals). One of the best examples is solidified mercury called sootha kattu.

Among various chunnam preparations a very few preparations are considered very indispensable as medicine and in medicine preparation, are termed as Guru chunnams. Very few dings like gold, silver, copper sulphate, fuller's earth chunnam preparations are considered as gold chunnam and termed as thanga guru, velli guru, thurusu guru, muppu guru respectively.

Among the many uniqueness of Siddha medicine, kayakalpam or rejuvenation therapy is its epitome. Food and life style modifications with ding therapy are indicated in this line of treatment. Muppu chunnam is basic and cardinal component in these preparations of anti ageing processes and also in alchemical transformations.

Apart from this, muppu chunnam is also used as an adjuvant for many medicaments for chronic degenerative diseases and is a purifier of metals and minerals.

Many chunnam preparations of sal ammoniac (*kambi navachara chunnam*).borneo camphor (*kaipoora chunnam*).copper sulphate (*thurisu*).arsenic *thalaga chunnam*:(*vanga chunnam*). copper *(thamira chunnam*), gold (*thanga churmami*. mercury (*rasa chunnam*). mercuric per chloride (*veera chunnam*). mica (*abrakam*). magnetic ore (*kantham iron - aya chunnam*) arsenic oxide sulphide (*gowri*). sulphur (*gandagam*). cinnabar (*lingam)*. zinc *(nagam*) are prepared with *muppu chunnam*

It is worth mentioning that very small quantity of copper sulphate *chunnam* (*thurusu*) is added to extract juices or latex from very dry natured plants like *virali*(Dodonea riscosa). *kuppai meni* (Acalipha indica) and *erukku(*Calotropis gigandica)

3.5. DISEASE REVIEW

KALLERAL NOI

3.5.1 SIDDHA ASPECT

Definition:

Damage of Liver cells or decrease or loss of Liver function or enlarge in size of Liver is called Liver disease.

Synomyms:

Valapattu eral noi,Manthakatti,Kalmantham,Yakkutham.

Aetiology:

- Excessive intake of food
- Alcoholism
- Obesity
- Associated with fever

Symptoms:

- Tastelessness
- Excessive of secretion of saliva
- Loss of appetite
- Indigestion
- Bile vomiting in morning
- Nausea,vomiting
- loss of weight
- Frequent fever
- Oedema in abdomen

Classification:

1 *Vali kalleral noi*

2 *Azhal kalleral noi*

3 *Aiya kalleral noi*

***Vali kalleral noi* symptoms:**

- Weight loss
- Blackish skin colour
- Ascites
- Enlarged lymph nodes
- Anemia with oedema

***Azhal kalleral noi* symptoms:**

- Decreased in Liver function
- Yellowish discolouration of the skin
- Bitter in taste
- Vomating
- Oedema
- Ascities

***Aiya kalleral noi* symptoms:**

- Itchy skin
- Swelling in the ankles and abdomen
- Abdomal pain & Bloating
- A brownish or orange - colour urine
- White stools
- Confusion disorientation, personality changes
- Blood in the stool
- Fever

MANJAL NOI

Definition:

Manjal noi refers to the yellowish discolouration of skin, Sclerae, mucos membrane and urine. Due to increased pitham and its complications.

The term kamalai is self explanatory refers to the meaning desirelessness or disinterested state due to any underlying cause whatever. The word kamalai is formed by the following two words.

Kamalai = kamam +illai

Therefore,there is no desire interest and ability neither with his daily activites nor with all other things including sex. Many causes can develop such condition. But Yugi out of his knowledge has classified this *kamalai* into 13 types as per the convenience of treatment method and symptomtology.

A group symptoms and signs when able to be treated by a group of medicine or a single medicine has been classified into a type. Thus according to predominant symptoms and therapeutic convenience 13 such types are classified by YUGI .V

Synonyms:

Pitha Noi, Manjal noi, kamalai, kamala, Kamila

Aeitology:

The three humors are responsible for the normal physiological conditions of the body.If any of these factors increase or decrease from its normal ratio.It leads to pathological changes in the body and thus produces disease.

According to “Yugi - 800” the *kamalai* is caused by the following conditions

“M÷‹ð«õ ð£‡´ ºŸP¼,°‹ «ð£¶

eP«ò Hˆîõv¶î¬ùŠ ¹Cˆî£™

¹÷‹ð«õ ñƒ¬è»ì¡ ¹í˜„C ªêŒî£™

Ì‡®´«ñ è£ñ£¬ô ªò¡Â‹ «ó£è‹”

1 Taking *pitha* substances when suffering from *pandu.*

2 Having excessive sexual intercourse.

3 Excessive intake of food.

4 Sleeplessness during in right time.

5 Excessive intake of highly spicy food items.

Signs and Symptoms:

"ð¼è«õ à œ÷ƒè£ ½œ÷ƒ ¬èèœ

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I,è£¶ ñ‰î‰î¬ô èùŠ¹‡ ì£°‹"

pallor of sole, palm, face, eyes and body.

Lassitude

Generalized malaise

Shivering

Dry stool and constipation

Yellow discolouration of face, oedema, fatiquability

Heaviness of head

Excessive salivation

Nausea

Bitter taste

Dryness of skin - frog like skin

Yellow discolouration of eye, nailbud, skin, tongue and urine.

Classification:

According to the medicine and convenience various *siddhars* have classified this disease ***kamalai*** in their own style.

In *'Thanvandri vaidhyam'kamalai* is classified into 5 types

In 'Agathiyar 2000' *kamalai* classified into 8 types another one is based on diseased wise

According to T.V Sambasivam pillai dictionary classified into 16 types

In *Balavagadam* it is classified into 3 kinds,

1.Oothu kamalai 2.Manjal kamalai 3.Varat kamalai

In our *Pothu maruthuvam,* it is classified into 13 types. They are,

1 *Vali kamalai*

2 *Uthu kamalai*

3 *Varat kamalai*

4 *Azal (or) Pitha kamalai*

5 *Aiya (or)kapa kamalai*

6 *Vadha kapa kamalai*

7 *Azhalaiya kamalai*

8 *Mukkutra kamalai*

9 *Azhaku kamalai*

10 *Senkamala kamalai*

11 *Manjal kamalai*

12 *Kunma kamalai*

13 *Kumba kamalai*

Naadi:

“ð ‡ ð£ù Hˆ îˆF™ «êˆ¶ñƒÃ® ðKCˆ î£™

è ‡ è£¶ ñôc¼ ñ…êœ....”

Management:

Purgative *(kalichal*)

Emesis (*vanthi)*

Avoid Tamarin and salt

Avoid oily and fatty foods

Add

Rice *kanji,* Tender coconut water, sugarcane juice, Tender vegetables, greens.

Dairy products

Cow’s milk, goat’s milk,

Non-vegetarian diets - *Ayirai meen* (loach).

3.5.2 MODERN ASPECT

LIVER DISEASES

Definition

Liver disease is any disturbance of liver function that cause illness. The liver is responsible for many critical functions within the body and should it become diseased or injured, the loss of those function can cause significant damage of the body. Liver disease is also reffered to as hepatic disease.

The liver can be damaged in a variety of ways

Cells can become inflamed for example hepatitis.

Bile flow can be obstructed for example cholestasis.

Cholesterol or triglycerides can accumulate for example steatosis.

Blood flow to liver may be compromised

Liver tissue can be damaged by chemicals and minerals or infiltrated by abnormal cells like cancer cells.

Liver disease comprise vast range of condition that affect the normal functioning of the liver. The symptoms related to the liver dysfunction include both physical signs and a variety of symptoms related to digestive problemcoagulapathies, blood sugar problems, immune disorder, abnormal absorption of fat and metabolism problems.

There are more than 100 kinds of liver disease like hepatitis, alcoholic disease, fatty live disease, cirrhosis hereditary disease, hepatic encephalopathy ,Acute liver failure, Haemochromatosis and liver cancer.

Cirrhosis of the liver:

Chronic liver damage from a variety of causes leading to scarring and liver failure.

Alcoholic hepatitis

Liver inflammation caused by drinking too much alcohol.

Hepatitis A

A highly contagious liver infection caused by the hepatitis A virus.

Hemo chromatosis

Hemochromatosis (iron overload) is a metabolic disorder that leads to abnormally evelated iron stores in the body. The excess iron may accumulate in the tissues of the liver, pancreas and heart and can lead to inflammation hemachromatasis is an inherited disease.

Alcohol abuse:

Alcohol is directly toxic to liver cells and can cause liver inflammation, hepatitis. In chronic alcohol abuse, fat accumulation occurs in liver cells affecting their ability to function.

Fatty liver develops in about 90% of individuals who drink more than 60mgs/day of alcohol. About 5% of the population can cause mild jaundice.

JAUNDICE

1 A medical condition with yellowing of the skin or whites of eyes, arising from excess of the pigment bilirubin and typically caused by obstruction of the bile duct by liver disease or by excessive breakdown of red blood cells.

2. Bitterness, resentment or cynicism.

3. It is also called icterus yellowing of the skin and white of the eyes due to the abnormal presence of bile pigments in the blood as in hepatitis.

Causes

Jaundice may be caused by several different disease processes. It is helpful to understand the different causes of jaundice by identifying the problems that disturb the normal bilirubin metabolism and or excretion.

Signs and Symptoms

Already mentioned Jaundice is not a disease but rather a visible sign of an underlying disease process.

Individuals with jaundice will have a yellow discoloration of the skin or varying degree and may also exhibit yellowing of the mucous membranes and the white of eyes.

Pale coloured - stools

Dark coloured - urine

Skin - itching

Nausea and vomiting , Rectal bleeding, Diarrhoea, Fever and chills, Weakness, weight loss, loss of appetite, confusion, abdominal pain headache swelling of the legs and swelling and distension of the abdomen.

Types of Jaundice

There are three main types of jaundice

Hepatocellular jaundice:

Yellowness of skin, sclera, mucous membranes and excretion due to hyperbilirubinemia and deposition of bile pigments.It is usually first noticeable in the eyes, although it may come on so gradually that is not immediately noticed by those in daily contact with the jaundiced person called also icterus.

Jaundice is not a disease;it is a symptom of a number of different diseases and disorders of the liver and gallbladder and of hemolytic blood disorders.One such disorder is the presence of a gallstone in the common bile duct, which carries bile from the liver to the intestine. This may obstruct the flow of bile, causing it to accumulate and enter the bloodstream. The obstruction of bile flow may cause bile to enter the urine, making it dark in colour, and also decrease the bile in the stool, making it light and clay-coloured. This condition requires surgery to remove the gallstone before it causes serious liver injury.

Obstructive jaundice:

Obstructive Jaundice is a particular type of jaundice and occurs when the essential flow of bile to the intestine is blocked and remains in the bloodstream. This might be due to blocked bile ducts caused by gall stones or tumours of the bile duct which can block the area where the bile duct meets the duodenum. These may be cancerous. Other conditions that can cause obstructive jaundice include those that cause pressure on the bile duct such as swelling of lymph glands scar tissue (from previous infections or surgery),or a cyst, possibly of the pancreas.

Hemolytic jaundice:

A type of jaundice that occurs as a result of hemolysis (an accelerated breakdown of erythrocytes- red blood cells) leading to an increase in production of bilirubin

Gall stone Formation

1 Gall stones are pieces of solid material that form in the gall bladder. These stones develop because cholesterol and pigments in bile.

2 Gall stones can form when there is an imbalance in the substance that make up bile for instance, cholesterolstones may develop as result of too much cholesterol in the bile.

3 Another cause may be the inability of the gallbladder to empty properly.

Although anyone can develop gallstones older and overweight people are more likely to get them further more women are at greater risk of developing gallstones.

4.MATERIALS AND METHODS

4.1 PREPARATION OF THE DRUG:

Selection of drug:

The Drug ***"Pavalaveerachunnam"*** has been selected for Hepatoprotective, Hypolipidemic, Diuretic and Lithotriptic activitities from the Classical Siddha literature ***"The pharmacopoeia of siddha research medicines "*** authored by Shanmugavelu.M.Dr**.,** Page no: 86.

Ingredients of the drug:

1. *Veera*m (Terlinguiate-Hg2clo)
2. *Pavalam* (Coral)- (Corallum rubrum.)
3. Goat bile

Collection of the drugs:

The raw material of *Veeram* was bought from the raw country drug shop at Nagercovil Kanniyakumari District, Tamilnadu.

The material Coral was bought from the raw country drug shop at Coimbathur, Kovai District, Tamilnadu.

Goat bile were collected from slater house, Tirunelveli market, Tamilnadu.

Identification and authentication:

All raw drugs were identified and Authenticated by the experts of *Geology* department in V.O.Chidambaram college Toothukudi, Tirunelveli District.

The specimen samples of the identified raw drugs were preserved in the laboratory of PG Gunapadam department in Govt.SiddhaMedical College,Palayamkottai for future references.

Purification of raw drugs:

Veeram - Tender coconut water and sufficient quantity of camphor mixed and poured into a pot. Veeram is packed with cotton cloth (Thulaenthiram) and suspended above the water level. It boiled for half an hour or until the water level reduced by $\frac{1}{3}$. The veeram washed out thoroughly and dried.

Pavalam - Pavalam is soaked in lemon juice for one day(24hrs).The next day it is washed with hot water and dried.

Preparation of *Pavalaveerachunnam*:

Ingredients of the drug ,

1. *Veeram* - 10gms (1part).
2. *Pavalam* (Coral) - 100gms (10part).
3. Goat Bile - Sufficient

Process:

Purified Pavalam and Veeram are broken into small pieces are powdered coarsely. Then put into mudpot (Kuduvai) and poured goat bile and stirred well and kept for a day. Next day exposed to the sunlight for drying.

Trituration: The above material was powdered well. Then it was triturated with Goatbile

***Villai*(small cakes) preparation and sealing:**

The mixture was made into small cakes (coin size). Then it dried under sunlight for two days. Then it placed in an earthen crucible and it is covered by internal earthen crucible. The margins are tightly sealed with a mud pasted cloth seven times and then it dried under sunlight for one day.

Incineration and trituration:

Then the earthen crucible was subjected to *pudam* (incineration) process with 250 cow dung cakes. After finishing *pudam* process let the earthen crucible undisturbed to give away heat. The seal was opened on the next day. The cakes of *Pavalaveeram* was collected and ground well. Finally *Chunnam* is obtained. The colour of the *Cunnam* is white in colour and the consistency was very fine.

Storage:

The drug *Pavalaveera chunnam* was stored in a clean air tight glass container and used for further studies.

Administration of the drug:

Form of the drug : Powder *(Chunnam)*

Route : Enteral*(oral)*

Dose : 65-130-mg (1-2 grains)

Adjuvant : Suitable adjuvant

Shelf life : 500 years.

4.2. STANDARDIZATION OF THE DRUG

4.2.1. AS PER *SIDDHA* CLASSICAL LITERATURE:

Standardization of drug means confirmation of it's quality and purity and detection of the nature of adulterant of various parameters like morphological, microscopic, physical, chemical and biological observations.

1. Colour:

The finished form of *Chunnam* is white in colour.

2. Odour:

The finished form of *Chunnam* is odourless, without any odour related to it's ingredients.

3. Taste:

A small amount of *Chunnam* was kept in the tip of the tongue, which is tasteless.Properly prepared *Chunnam* should be completely tasteless. If any taste present in *Chunnam,* it indicates the preparation was not well prepared. It needs another *pudam*(incineration) process.

4. Finger Print Test:

Well prepared *Chunnams*hould be very fine. A pinch of *Chunnam*was taken and rubbed in between the thumb and index finger. It entered into the depressions and furrows of the fingers. Itconfirmes the fineness of *Chunnam.*

5. Floating on Water:

A pinch of *Chunnam*was sprinkled over the water in a glass container. The *Chunnam*particles did not sink but floated on the water surface. It indicates the lightness of *Chunnam*.

6. Lustre:

If any glowing particles seen in the *Chunnam,* it shows that the drug is not prepared properly and possess unchanged substances like metals and other toxic substances. So, there should be no glowing particles present in the properly prepared *Chunnam.* The *Chunnam* was taken in a Petri dish and observed for any lustre in daylight via magnifying glass.

7.Colour change

The chunnam was mixed with termaric powder. The yellow colour was changed into red colour

4.2.2. STANDARDIZATION OF TEST DRUG BY USING MODERN TECHNIQUES:

Standardization of drug helps to authenticate and determine its quality and efficiency. Thus, the process involves qualitative and quantitative analysis by means of physico – chemical properties and instrumental analysis.

The physic-chemical analysis of *Pavalaveerachunnam* has been done in Aravind Herbal Labs (P) Ltd., Rajapalayam.

The chemical finger prints are engaged by using modern analytical technique Fourier Transform Infra –Red Spectroscopy (FTIR) and Inductively coupled plasma mass spectrometry(ICP-MS)

The particle size and qualitative analysis of chemical elements of pavalaveerachunnam assessed by Scanning Electron Microscope (SEM) and Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES) and Inductively coupled plasma(ICP-MS)

4.2.2.1 PHYSICO CHEMICAL ANALYSIS:

Solubility test:

A. A little amount of the sample was taken in a dry test tube and shaken well with distilled water.

B. A little amount of the sample was taken in a clean dry test tube and then shaken well with con.HCl and Con.H2SO4. Sparingly soluble character of the sample indicates the presence of Silicate.

Action on heat:

A small amount of the sample was taken in a clean dry test tube and heated gently. If strong white fumes evolve indicate the presence of Carbonate.

Flame test:

A small amount of the sample was taken in a clean dry watch glass. It was made into paste with concentrated HCL. And then it was introduced into non-luminous part of the Bunsen flame. If bluish green flame appears, it indicates the presence of copper.

Ash test:

A small amount of sample was mixed with the cobalt nitrate solution. A filter paper was soaked into the mixture. Then it was introduced into the Bunsen flame and ignited. If yellow colour flame appears, it reveals the presence of sodium.

Determination of Total Ash:

Incinerate about 2 to 3 g accurately weighed, of the ground drug in a tared platinum or silica dish at a temperature not exceeding 450º until free from carbon, cool and weigh. If a carbon free ash cannot be obtained in this way, exhaust the charred mass with hot water, collect the residue on an ashless filter paper, incinerate the residue and filter paper, add the filtrate, evaporate to dryness, and ignite at a temperature not exceeding 450º. Calculate the percentage of ash with reference to the air-dried drug.

Determination of Acid Insoluble Ash:

Boil the ash obtained in above test for 5 minutes with 25 ml of dilute hydrochloric acid; collect the insoluble matter in a Gooch crucible, or on an ashlessfilter paper, wash with hot water and ignite to constant weight. Calculate the percentage of acid-insoluble ash with reference to the air dried drug.

Determination of Moisture Content (Loss on Drying):

Procedure set forth here determines the amount of volatile matter (i.e., water drying off from the drug). For substances appearing to contain water as the only volatile constituent, the procedure given below, is appropriately used.

Place about 10 g of drug (without preliminary drying) after accurately weighing (accurately weighed to within 0.01 g) it in a tared evaporating dish. For example, for underground or unpowdered drug, prepare about 10 g of the sample by cutting shredding so that the parts are about 3 mm in thickness.

Seeds and fruits, smaller than 3 mm should be cracked. Avoid the use of high speed mills in preparing the samples, and exercise care that no appreciable amount of moisture is lost during preparation and that the portion taken is representative of the official sample. After placing the above said amount of the drug in the tared evaporating dish dry at 105º for 5 hours, and weigh. Continue the drying and weighing at one hour interval until

difference between two successive weighings corresponds to not more than 0.25 per cent. Constant weight is reached when two consecutive weighings after drying for 30 minutes and cooling for 30 minutes in a desiccator, show not more than 0.01 g difference.

MICROBIAL LIMIT TESTS:

Determination of Total Aerobic Microbial Count:

Dissolve 10 g or dilute 10 ml of the preparation being examined, unless otherwise specified, in buffered sodium chloride-peptone solution Ph 7.0 or any other suitable medium shown to have no antimicrobial activity under the conditions of test and adjust the volume to 100 ml with the same medium. If necessary, adjust the pH to about 7.

Membrane filtration:

Use membrane filters 50 mm in diameter and having a nominal pore size not greater than 0.45 μm the effectiveness of which in retaining bacteria has been established for the type of preparation being examined. Sterilise and assemble the filtration apparatus described under tests for sterility.

Transfer 10 ml or a quantity of each dilution containing 1 g of the preparation being examined to each of two membrane filters and filter immediately. If necessary, dilute the pretreated preparation so that a colony count of 10 to 100 may be expected. Wash each membrane by filtering through it three or more successive quantities, each of about 100 ml, of a suitable liquid such as buffered sodium chloride-peptone solution pH 7.0. For fatty substances add to the liquid polysorbate 20 or polysorbate 80. Transfer one of the membrane filters, intended for the enumeration of bacteria, to the surface of a plate of casein soyabean digest agar and the other, intended for the enumeration of fungi, to the surface of a plate of Sabouraud dextrose agar with antibiotics.

Incubate the plates for 5 days, unless a more reliable count is obtained in shorter time, at 30º to 35º in the test for bacteria and 20º to 25º in the test for fungi. Count the number of colonies that are formed. Calculate the number of micro-organisms per gm or per ml of the preparation being examined, if necessary counting bacteria and fungi separately.

Plate count: For bacteria:

Using Petri dishes 9 to 10 cm in diameter, add to each dish a mixture of 1 ml of the pretreated preparation and about 15 ml of liquefied casein soyabean digest agar at not more than 45º. Alternatively, spread the pretreated preparation on the surface of the solidified medium in a Petri dish of the same diameter. If necessary, dilute the pretreated preparation as described above so that a colony count of not more than 300 may be expected. Prepare at least two such Petri dishes using the same dilution and incubate at 30º to 35º for 5 days, unless a more reliable count is obtained in a shorter time. Count the number of colonies that are formed. Calculate the results using plates with the greatest number of colonies but taking 300 colonies per plate as the maximum consistent with good evaluation.

For fungi:

Proceed as described in the test for bacteria but use Sabouraud dextrose agar with antibiotics in place of casein soyabean digest agar and incubate the plates at 20º to 25º for 5 days, unless a more reliable count is obtained in a shorter time. Calculate the results using plates with not more than 100 colonies.

TESTS FOR SPECIFIED MICRO-ORGANISMS:

Pretreatment of the sample being examined – Proceed as described under the test for total aerobic microbial count but using lactose broth or any other suitable medium shown to have no antimicrobial activity under the conditions of test in place of buffered sodium chloride-peptone solution pH 7.0.

Escherichia coli:

Place the prescribed quantity in a sterile screw-capped container, add 50 ml of nutrient broth, shake, allow to stand for 1 hour (4 hours for gelatin) and shake again. Loosen the cap and incubate at 37º for 18 to 24 hours.

Primary test:

Add 1.0 ml of the enrichment culture to a tube containing 5 ml of MacConkey broth. Incubate in a water-bath at 36º to 38º for 48 hours. If the contents of the tube show acid and gas carry out the secondary test.

Secondary test:

Add 0.1 ml of the contents of the tubes containing (a) 5 ml of MacConkey broth, and (b) 5 ml of peptone water. Incubate in a water-bath at 43.5º to 44.5º for 24 hours and examine tube (a) for acid and gas and tube (b) for indole. To test for indole, add 0.5 ml of Kovac's reagent, shake well and allow to stand for 1 minute; if a red colour is produced in the reagent layer indole is present. The presence of acid and gas and of indole in the secondary test indicates the presence of Escherichia coli.

Carry out a control test by repeating the primary and secondary tests adding 1.0 ml of the enrichment culture and a volume of broth containing 10 to 50 Escherichia coli (NCTC 9002) organisms, prepared from a 24-hour culture in nutrient broth, to 5 ml of MacConkey broth. The test is not valid unless the results indicate that the control contains Escherichia coli.

Salmonella:

Transfer a quantity of the pretreated preparation being examined containing 1 gm or 1 ml of the product to 100 ml of nutrient broth in a sterile screwcapped jar, shake, allow to stand for 4 hours and shake again. Loosen the cap and incubate at 35º to 37º for 24 hours.

Primary test:

Add 1.0 ml of the enrichment culture to each of the two tubes containing (a) 10 ml of selenite F broth and (b) tetrathionate-bile-brilliant green broth and incubate at 36º to 38º for 48 hours. From each of these two cultures subculture on at least two of the following four agar media: bismuth sulphate agar, brilliant green agar, desoxycholatecitrate agar and xylose-lysine-desoxycholate agar. Incubate the plates at 36º to 38º for 18 to 24 hours. Upon examination, if none of the colonies conforms to the description given in Table 2, the sample meets the requirements of the test for the absence of the genus Salmonella.

If any colonies conforming to the description in Table 3 are produced, carry out the secondary test.

Secondary test:

Subculture any colonies showing the characteristics given in Table 3 in triple sugar-iron agar by first inoculating the surface of the slope and then making a stab culture with the same inoculating needle, and at the same time inoculate a tube of urea broth. Incubate at 36º to 38º for 18 to 24 hours. The formation of acid and gas in the stab culture (with or without concomitant blackening) and the absence of acidity from the surface growth in the triple sugar iron agar, together with the absence of a red colour in the urea broth, indicate the presence of salmonellae. If acid but no gas is produced in the sub culture, the identity of the organisms should be confirmed by agglutination tests.

Carry out the control test by repeating the primary and secondary tests using 1.0 ml of the enrichment culture and a volume of broth containing 10 to 50 Salmonella abony (NCTC 6017) organisms, prepared from a 24-hour culture in nutrient broth, for the inoculation of the tubes (a) and (b). The test is not valid unless the results indicate that the control contains Salmonella.

Table No. 1.Tests for Salmonella

Medium	**Description of colony**
Bismuth Sulphite agar	Black or green
Brilliant green agar	Small, transparent and colorless, or opaque, pinkish or white (frequently surrounded by a pink or red zone)
Deoxycholate – Citrate agar	Colourless and opaque, with or without black centers
Xylose-lysine-desoxy-cholate agar	Red with or without black centers

Pseudomonas aeruginosa:

Pretreat the preparation being examined as described above and inoculate 100 ml of fluid soyabean-casein digest medium with a quantity of the solution, suspension or emulsion thus obtained containing 1 g or 1 ml of the preparation being examined. Mix and incubate at 35º to 37º for 24 to 48 hours. Examine the medium for growth and if growth is present, streak a portion of the medium onthe surface of cetrimide agar medium, each

plated on Petri dishes. Cover and incubate at 35º to 37º for 18 to 24 hours. If, upon examination, none of the plates contains colonies having the characteristics listed in Table 4 for the media used, the sample meets the requirement for freedom from Pseudomonas aeruginosa. If any colonies conforming to the description in Table 4 are produced, carry out the oxidase and pigment tests.

Streak representative suspect colonies from the agar surface of cetrimide agar on the surfaces of pseudomonas agar medium for detection of fluorescein and pseudomonas agar medium for detection of pyocyanin contained in Petri dishes. Cover and invert the inoculated media and incubate at 33º to 37º for not less than 3 days. Examine the streaked surfaces under ultra-violet light. Examine the plates to determine whether colonies conforming to the description in Table 4 are present.

If growth of suspect colonies occurs, place 2 or 3 drops of a freshly prepared 1% w/v solution of N, N, N1, N1-tetramethyl-4-phenylenediamine dihydrochloride on filter paper and smear with the colony; if there is no development of a pink colour, changing to purple, the sample meets the requirements of the test for the absence of Pseudomonas aeruginosa.

Table No. 2. Tests for Pseudomonas aeruginosa

Medium	**Characteristic colonical morphology**	**Fluorescence in UV light**	**Oxidase test**	**Gram stain**
Cetrimde agar	Generally greenish	Greenish	Positive	Negative rods
Pseudomonas agar medium for detection of fluorescein	Generally colourless to yellowish	Yellowish	Positive	Negative rods
Pseudomonas agar medium for detection of pyocyanin	Generally greenish	Blue	Positive	Negative rods

Staphylococcus aureus:

Proceed as described under Pseudomonas aeruginosa. If, upon examination of the incubated plates, none of them contains colonies having the characteristics listed in Table 5 for the media used, the sample meets the requirements for the absence of Staphylococcus aureus.

If growth occurs, carry out the coagulase test. Transfer representative suspect colonies from the agar surface of any of the media listed in Table 5 to individual tubes, each containing 0.5 ml of mammalian, preferably rabbit or horse, plasma with or without additives. Incubate in water-bath at 37º examining the tubes at 3 hours and subsequently at suitable intervals up to 24 hours. If no coagulation in any degree is observed, the sample meets the requirements of the test for the absence of Staphylococcus aureus.

Table No. 3.Tests for Staphylococcus aureus

Selective medium	**Characteristic colonical morphology**	**Gram stain**
Vogel-Johnson agar	Black surrounded by yellow zones`	Positive cocci (in clusters)
Mannitol-salt agar	Yellow colonies with yellow zones	Positive cocci (in clusters)
Baired-Parker agar	Black, shiny, surrounded by clear zones of 2 to 5 mm	Positive cocci (in clusters)

Thin-Layer Chromatography (TLC)

Thin-layer chromatography is a technique in which a solute undergoes distribution between two phases, stationary phase acting through adsorption and a mobile phase in the form of a liquid. The adsorbent is a relatively thin, uniform layer of dry finely powdered material applied to a glass, plastic or metal sheet or plate. Glass plates are most commonly used. Separation may also be achieved on the basis of partition or a combination of partition and adsorption, depending on the particular type of support, its preparation and its use with different solvent.

Identification can be effected by observation of spots of identical Rf value and about equal magnitude obtained, respectively, with an unknown and a reference sample chromatographed on the same plate. A visual comparison of the size and intensity of the spots usually serves for semi-quantitative estimation.

Apparatus

a) Flat glass plates of appropriate dimensions which allow the application at specified points of the necessary quantities of the solution being examined and appropriate reference solutions and which allow accommodation of the specified migration path-length. The plates are prepared as described below; alternatively, commercially prepared plates may be used.

b) An aligning tray or a flat surface on which the plates can be aligned and rested when the coating substance is applied.

c) The adsorbent or coating substance consisting of finely divided adsorbent materials, normally 5ìm to 40ìm in diameter is suitable for chromatography. It can be applied directly to the plate or can be bonded to the plate by means of Plaster of Paris (Hydrated Calcium Sulphate) or with any other suitable binders. The adsorbent may contain fluorescing material to help in visualizing spots that absorb ultra-violet light.

d) A spreader which, when moved over the glass plate, will apply a uniform layer of adsorbent of desired thickness over the entire surface of the plate.

e) A storage rack to support the plates during drying and transportation.

f) A developing chamber that can accommodate one or more plates and can be properly closed and sealed. The chamber is fitted with a plate support rack that supports the plates, back to back, with lid of the chamber in place.

g) Graduated micro-pipettes capable of delivering microlitre quantities say 10ìl and less.

h) A reagent sprayer that will emit a fine spray and will not itself be attacked by the reagent.

i) An ultra-violet light, suitable for observation at short (254 nm) and long (365 nm) ultra-violet wavelengths.

Preparation of plates:

Unless otherwise specified in the monograph, the plates are prepared in the following manner. Prepare a suspension of the coating substance in accordance with the instructions of the supplier and, using the spreading device designed for the purpose, spread a uniform layer of the suspension, 0.25 to 0.30 mm thick, on a flat glass plate 20 cm long. Allow the coated plates to dry in air, heat at 100º to 105º for at least 1 hour (except in the case of plates prepared with cellulose when heating for 10 minutes is normally sufficient) and allow to cool, protected from moisture. Store the plates protected from moisture and use within 3 days of preparation. At the time of use, dry the plates again, if necessary, as prescribed in the monographs.

Method

Unless unsaturated conditions are prescribed, prepare the tank by lining the walls with sheets of filter paper; pour into the tank, saturating the filter paper in the process, sufficient of the mobile phase to form a layer of solvent 5 to 10 mm deep, close the tank and allow to stand for 1 hour at room temperature. Remove a narrow strip of the coating substance, about 5 mm wide, from the vertical sides of the plate. Apply the solutions being examined in the form of circular spots about 2 to 6 mm in diameter, or in the form of bands (10 to 20 mm x 2 to 6 mm unless otherwise specified) on a line parallel with, and 20 mm from, one end of the plate, and not nearer than 20 mm to the sides; the spots should be 15 mm apart. If necessary, the solutions may be applied in portions, drying between applications. Mark the sides of the plate 15 cm, or the distance specified in the monograph, from the starting line. Allow the solvent to evaporate and place the plate in the tank, ensuring that it is as nearly vertical as possible and that the spots or bands are above the level of the mobile phase. Close the tank and allow to stand at room temperature, until the mobile phase has ascended to the marked line. Remove the plate and dry and visualise as directed in the monograph; where a spraying technique is prescribed it is essential that the reagent be evenly applied as a fine spray.

For two-dimensional chromatography dry the plate after the first development and carry out the second development in a direction perpendicular to the first.

When the method prescribed in the monograph specified 'protected from light' or 'in subdued light' it is intended that the entire procedure is carried out under these conditions.

Visualisation

The phrases ultra-violet light (254 nm) and ultra-violet light (365 nm) indicate that the plate should be examined under an ultra-violet light having a maximum output at about 254 or at about 365 nm, as the case may be.

The term secondary spot means any spot other than the principal spot. Similarly, a secondary band is any band other than the principal band.

Rf. Value

Measure and record the distance of each spot from the point of its application and calculate the Rf. value by dividing the distance travelled by the spots by the distance travelled by the front of the mobile phase.

4.2.2.2 CHEMICAL ANALYSIS:

Preliminary Basic and Acidic Radical Studies:

Preparation of the Extract:

100mg of TPP is weighed accurately and placed into a clean beaker and added a few drops of conc. Hydrochloric acid and evaporated it well. After evaporation cooled the content and added a few drops of conc. Nitric acid and evaporated it well. After cooling the content add 20ml of distilled water and dissolved it well. Then it is transferred to 100ml with distilled water and stirred well. Filter it. Then it is taken for analysis.

Qualitative Analysis For Basic Radicals:

Test for Calcium:

2ml of the above prepared extract is taken in a clean test tube. To this add 2ml of 4% Ammonium oxalate solution. Formation of white precipitate indicates the presence of calcium.

Test for Iron (Ferric):

The extract is acidified with glacial acetic acid and potassium ferro cyanide. Formation of blue colour indicates the presence of ferric iron.

Test for Iron (Ferrous):

The extract is treated with concentrated Nitric acid and ammonium thio-cyanate solution. Formation of blood red colour indicates the presence of ferrous iron.

Test for Zinc:

The extract is treated with potassium ferro-cyanide. Formation of white precipitate indicates the presence of zinc.

Qualitative Analysis for Acidic Radicals:

Test for Sulphate:

2ml of extract is added to 5% barium chloride solution. Formation of white precipitate indicates the presence of sulphate.

Test for Chloride:

The extract is treated with silver nitrate solution. Formation of white precipitate indicates the presence of chloride.

Test for Phosphate:

The extract is treated with ammonium molybdate and concentrated nitric acid. Formation of yellow precipitate indicates the presence of phosphate.

Test for Carbonate:

On treating the extract with concentrated hydrochloric acid giving brisk effervescence indicates the presence of carbonate.

Test for starch:

The extract is added with weak iodine solution. Formation of blue colour indicates the presence of starch.

Test for albumin:

The extract is treated with Esbach‘s reagent. Formation of yellow precipitate indicates the presence of albumin.

Test for tannic acid:

The extract is treated with ferric chloride. Formation of bluish black precipitate indicates the presence of tannic acid.

Test for unsaturation:

The extract is treated with potassium permanganate solution. The dis-colourization of potassium permanganate indicates the presence of unsaturated compounds.

Test for the reducing sugar:

5ml of Benedict‘s qualitative solution is taken in a test tube and allowed to boil for 2 minutes and added 8-10 drops of the extract and again boil it for 2 minutes. Any colour change indicates the presence of reducing sugar.

Test for aminoacid:

One or two drops of the extract is placed on a filter paper and dried it well. After drying, 1% Ninhydrin is sprayed over the same and dried it well. Formation of violet colour indicates the presence of amino acid.

Test for mercury:

The extract is heated with Ammonia and boil (still the ammonia caeses off) and then potassium iodide (1% solution) is added .No scarlet precipitate is formed.It indicates the absence of mercury.

4.2.2.3 INSTRUMENTAL ANALYSIS

SCANNING ELECTRON MICROSCOPE (SEM)

Figure No 4: HITACHI S-3400H SEM ANALYSER

The microstructure of the powders was examined using a Hitachi S 3000H scanning electron microscope (Fig.12). The scanning Electron Microscope is one of the most versatile instruments available for the examination and analysis of the micro structural characteristics of solid objects. The primary reason for the SEM's usefulness is the high resolution which can be obtained when bulk objects are examined; values of the order of 5nm (50degreeA) are usually quoted for commercial instruments. Advanced research instruments have been described which have achieved resolutions of about 2.5nm (25 degree A). Any solid material can be studied. Sample size is limited to specimens less than about 10μm in diameter. An electron beam passing through an evacuated column is focused by electromagnetic lenses onto the specimen surface. The beam is then rastered 0ver the specimen in synchronism with the beam of a cathode ray tube display screen. In eleastically scattered secondary electrons are emitted from the sample surface and collected by a scintillator, the signal from which is used to modulate the brightness of the cathode ray tube. In this way the secondary electron emission from

the sample is used to form an image on the CRT display screen. (Goldstein, et. al., 1992)

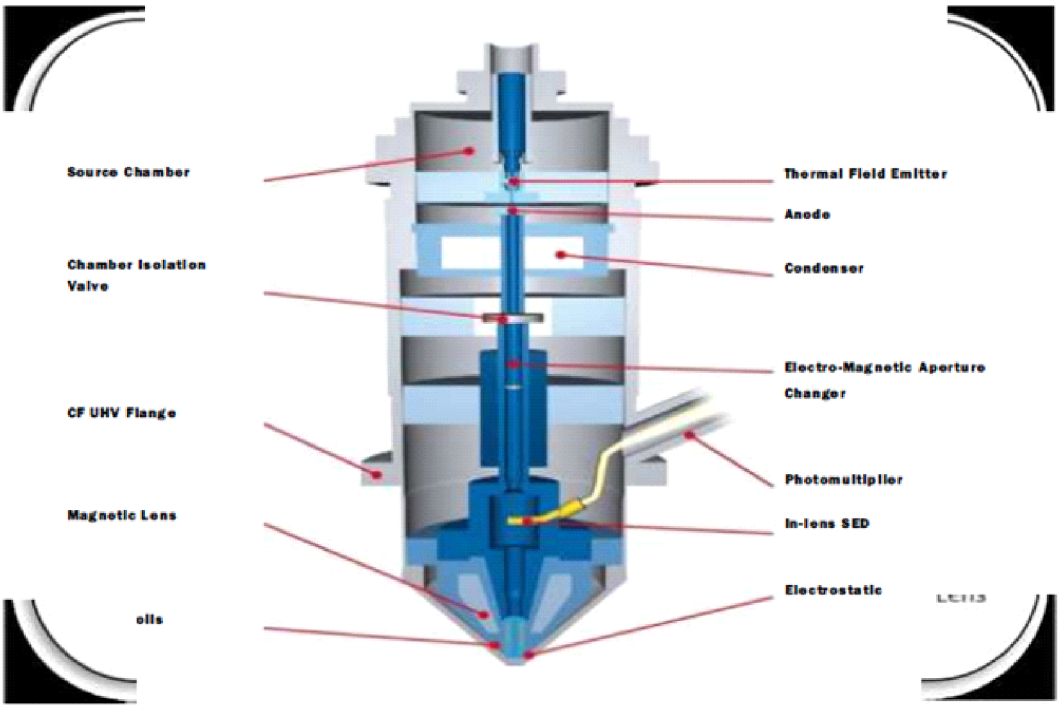


Figure No 5: MECHANISM OF SEM ANALYSER

Differences in secondary emission result from changes in surface topography.

If (elastically) back-scattered electrons are collected to form the image, contrast results

from compositional differences. Cameras are provided to record the images on the display screen. Since an electron is a charged particle, it has a strong interaction with the specimen (due to coulomb interaction). When an electron beam images on a specimen, it is scattered by atomic layers near the surface of the specimen. As a result, the direction of electron motion changes and its energy is partially lost. Once an incident electron (primary electron)enters a substance, its direction of motion is influenced by various obstructions (multiple scattering), and follows a complicated trajectory which is far from a straight line. Also, when electrons with the same energy are incident on the specimen surface, a portion of electrons is reflected in the opposite direction (back scattered) and the remainder is absorbed by the specimen (exciting X- rays or other quanta in the process). If the specimen

is sufficiently thin, the electron can pass all the way through the specimen (transmitted electrons, scattered or non-scattered).

The depth at which various signals are generated due to electron beam – specimen interaction indicates the diffusion area of the signals in the specimen in addition to the local chemistry of the specimen. Secondary electrons mainly indicate information about the surface of a specimen. Since secondary electrons do not diffuse much inside the specimen, they are most suitable for observing the fine-structures of the specimen surface. That is to say, sharp scanning images with high resolution can be expected from secondary electrons, because of the smaller influence on resolution by their diffusion.

As the incident electron energy increases, the probability of incident electrons colliding with elemental components of the specimen and releasing secondary electrons also increases. In other words, as the incident energy increases, the emission of electrons from the specimen also increases. However, as the energy increases beyond a certain level, the incident electrons penetrate deeper into the specimen with the result that the specimen derived electrons use up most of their energy to reach the specimen surface. Consequently, the electron emission yield decreases. Therefore, the peak secondary electron emission yield occurs at a specific entry level of the incident electrons.

In order to verify the existence of a substance and recognize its shape, the image contrast must be well defined. In other words, even if a system boasts extremely high resolution, if image contrast is poor, it would be extremely difficult to determine the existence of a substance, let alone recognize its shape. Another important feature of the SEM is the three-dimensional appearance of the specimen image, which is a direct result of the large depth of field. The SEM is also capable of examining objects at very low magnification. This feature is useful in viewing particle size and shape of any composition at various stages of preparation in siddha system as well as other fields.

The large depth of field available in the SEM makes it possible to observe 3-dimensional objects in stereo. Today, a majority of SEM facilities are equipped with X-ray analytical capabilities. Thus topographic crystallographic and compositional information can be obtained rapidly, efficiently and simultaneously from the same area.

The author was chosen this analysis for detecting Particle size of the classical siddha mineral drug ***Pavalaveerachunnam.*** SEM results of ***Pavalaveerachunnam*** was represented in results section.

INDUCTIVELY COUPLED PLASMA - OPTICAL EMISSION SPECTROMETRY (ICP-OES)

Figure No 6: IMAGE OF ICP-OES

Inductively coupled plasma optical emission spectrometry (ICP-OES) is an analytical technique used for the detection of trace metals. It is a type of emission spectroscopy that uses the inductively coupled plasma to produce excited atoms and ions that emit electromagnetic radiation at wavelengths characteristic of a particular element. The intensity of this emission is indicative of the concentration of the element within the sample.

Mechanism

The ICP-OES is composed of two parts: the ICP and the optical spectrometer. The ICP torch consists of 3 concentric quartz glass tubes. The output or “work” coil of the

radiofrequency (RF) generator surrounds part of this quartz torch. Argon gas is typically used to create the plasma.

When the torch is turned on, an intense electromagnetic field is created within the coil by the high power radio frequency signal flowing in the coil. This RF signal is created by the RF generator which is, effectively, a high power radio transmitter driving the "workcoil" the same way a typical radio transmitter drives a transmitting antenna. The argon gas flowing through the torch is ignited with a Tesla unit that creates a brief discharge arc through theargon flow to initiate the ionization process. Once the plasma is "ignited", the Tesla unit is turned off.

The argon gas is ionized in the intense electromagnetic field and flows in a particular rotationally symmetrical pattern towards the magnetic field of the RF coil. A stable, high temperature plasma of about 7000 K is then generated as the result of the inelastic collisions created between the neutral argon atoms and the charged particles. A peristaltic pump delivers an aqueous or organic sample into a nebulizer where it is changed into mist and introduced directly inside the plasma flame. The sample immediately collides with the electrons and charged ions in the plasma and is itself broken down into charged ions. The various molecules break up into their respective atoms which then lose electrons and recombine repeatedly in the plasma, giving off radiation at the characteristic wavelengths of the elements involved.

Within the optical chamber(s), after the light is separated into its different wavelengths (colors), the light intensity is measured with a photomultiplier tube or tubes physically positioned to "view" the specific wavelength(s) for each element line involved , or, in more modern units, the separated colors fall upon an array of semiconductor photo detectors such as charge coupled devices (CCDs). In units using these detector arrays, the intensities of all wavelengths (within the system's range) can be measured simultaneously ,allowing the instrument to analyze for every element to which the unit is sensitive all at once. Thus, samples can be analyzed very quickly.

The intensity of each line is then compared to previously measured intensities ofknown concentrations of the elements, and their concentrations are then computed by interpolation along the calibration lines. In addition, special software generally corrects

for interferences caused by the presence of different elements within a given sample matrix. Examples of the application of ICP-OES include the determination of metals, arsenic present in Traditional medicines, and trace elements bound to proteins. ICP-OES is widely used in minerals processing to provide the data on grades of various streams, for the construction of mass balances.

The author used for elemental identification and quantitative compositional information of the ***Pavalaveerachunnam***

FOURIER TRANSFORM - INFRA RED SPECTROSCOPY (FT-IR)

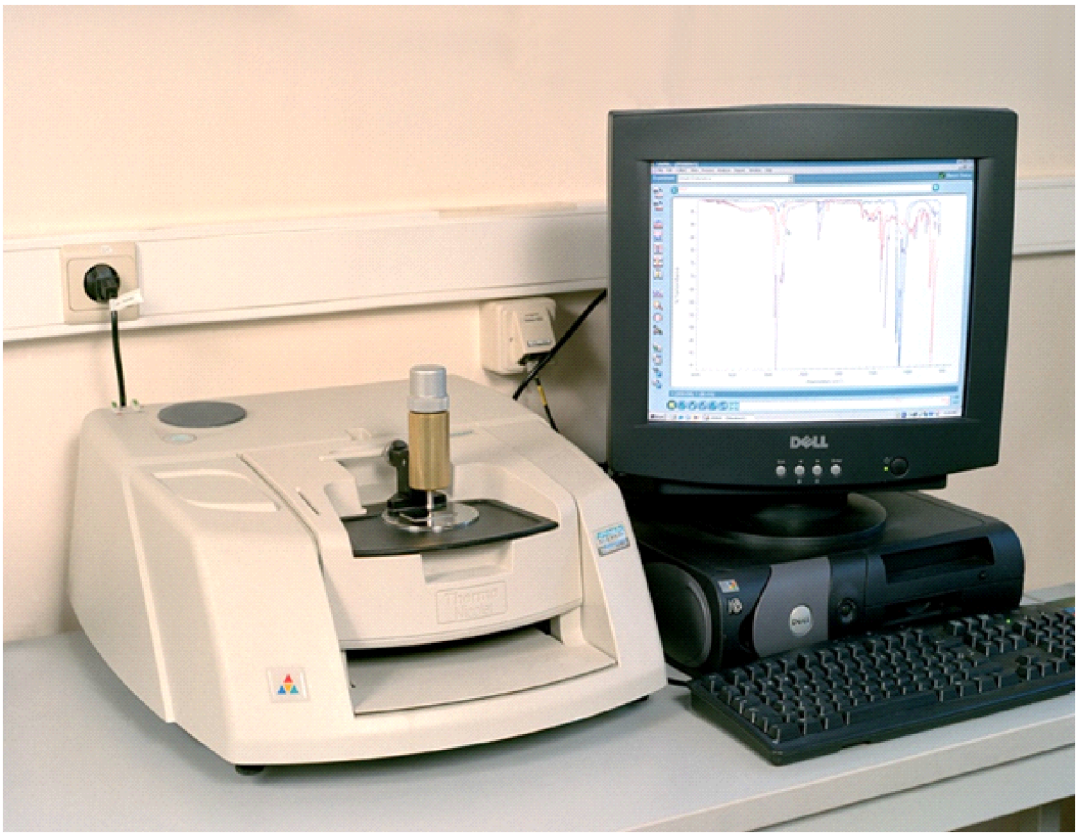


Figure No 7: FTIR-SPECTRUM ANALYSER

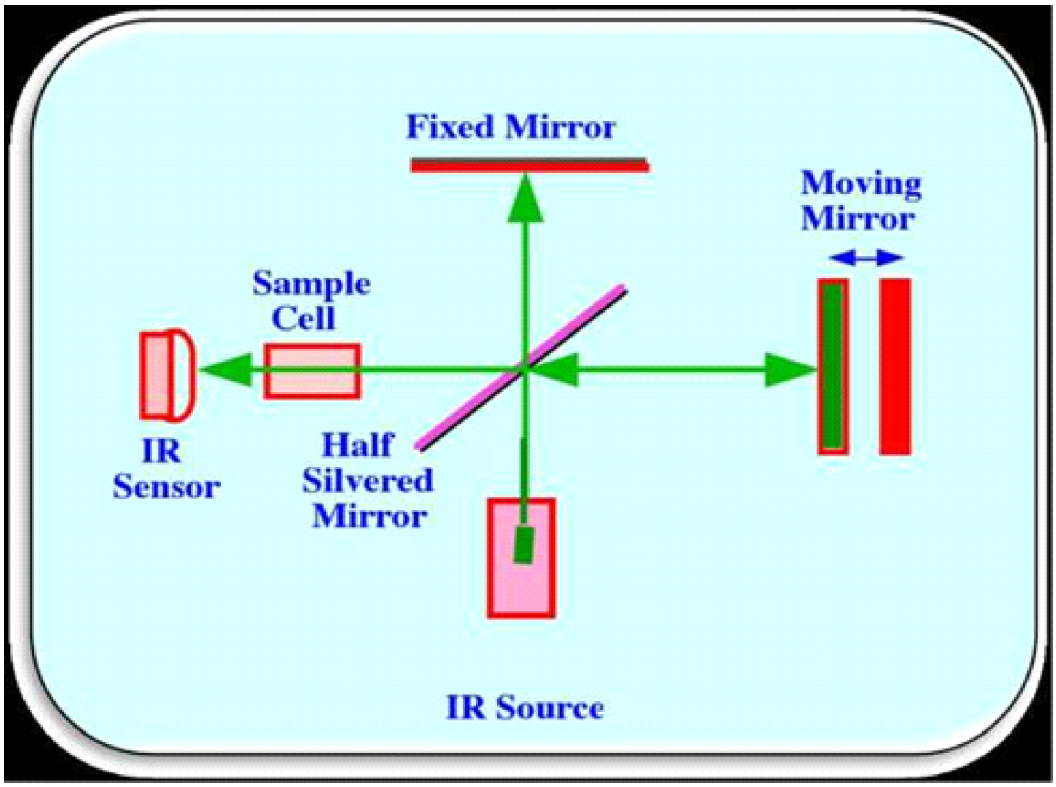


Figure No 8: MECHANISM OF FTIR SAMPLING TECHNIQUES

Introduction

Vibrational spectroscopy is an extremely useful tool in the elucidations of molecular structure. The spectral bands can be assigned to different vibrational modes of the molecule. The various functional groups present in the molecule can be assigned by a comparison of the spectra with characteristic functional group frequencies. As the positions of the bands are directly related to the strength of the chemical bond, a large number of investigations including intermolecular interactions, phase transitions and chemical kinetics can be carried out using this branch of spectroscopy. In IR spectroscopy, the resonance absorption is made possible by the change in dipole moment accompanying the vibrational transition. The Infrared spectrum originates from the vibrational motion of the molecule. The vibrational frequencies are a kind of fingerprint of the compounds. This property is used for characterization of organic, inorganic and biological compounds. The band intensities are proportional to the concentration of the compound and hence qualitative estimations are possible. The IR spectroscopy is carried out by using Fourier transform technique.

Principle

Infra red spectroscopy involves study of the interaction of electromagnetic radiation with matter. Due to this interaction, electromagnetic radiation characteristic of the interacting system may be absorbed (or emitted). The experimental data consist of the nature (frequency of wave length) and the amount (intensity) of the characteristic radiation absorbed or emitted. These data are correlated with the molecular and electronic structure of the substance and with intra- and inter molecular interactions.

Source	**:**	Nernst Glower
Beam splitter	**:**	It is made up of a transparent material. Thin films ofSilicondeposited on Potassium bromide (KBr)
Bromide (KBr) Detectors	:	DeutratedTriGlycineSulphate (DTGS).
MIR Range	:	4000 to 450 cm-1
Resolution	:	4.0 cm-1

Sampling Techniques

There are a variety of techniques for sample preparation depending on the physical form of the sample to be analyzed.

Solid	:	KBr or Nujol mull method.
Liquid	:	Csl / TIBr Cells
Gas	:	Gas cells

KBr Method

- The sample is grounded using an agate mortar and pestle to give a very fine powder.
- The finely powder sample is then mixed with about 100mg dried KBr salt.
- The mixture is then pressed under hydraulic press using a die to yield a transparent disc and measure about 13mm diameter and 0.3mm in thickness.

Nujol Mull Method

- The sample is ground using an agate mortar and pestle to give a very fine powder.

- A small amount is then mixed with nujol oil to give a paste and this paste is then applied between two sodium chloride plates.
- The plates are then placed in the instrument sample holder ready for scanning.

Liquids

- Viscous liquids can be smeared in the cell and directly measured.
- For dilute solutions, liquid cells and variable path length cells are employed.

Measurements Techniques

The procedure for recording the %T or %A is as follows:

- Air is first scanned for the reference and stored. The sample is then recorded and finally the ratio of the sample and reference data is computed to give required %T or %A at various frequencies.
- Study of substances with strong absorbance bands and weak absorbance bands aswell as possible.
- Small amount of samples are sufficient
- High resolution is obtained.

Procedure

- Preparation of samples for infrared measurements and infrared spectra Typically,1.5 mg of protein, dissolved in the buffer used for its purification, were centrifuged in a 30 K Centric on micro concentrator (Amicon) at 3000_g at 4oC until a volume of approximately 40 Al.
- Then, 300 Al of 20 mMThis buffer, prepared in H20 or 2H20, pH or p2H 7.2, were added and the sample concentrated again. The p2H value corresponds to the pH meter reading + 0.4. The concentration and dilution procedure was repeated several times in order to completely replace the original buffer with the Tris buffer.
- The washings took 24 h, which is the time of contact of the protein with the 2H20 medium prior FT-IR analysis. In the last washing, the protein was concentrated to fine a volume of approximately 40 Al and used for the infrared measurements.

- The concentrated protein sample was placed in CaF2 windows and a 6 Am tin spacer or a 25 Am Teflon spacer for the experiments in H20 or 2H20, respectively. FT-IR spectra were recorded by means of a Perkin-Elmer -Spectrum-1 FT-IR spectrometer using a deuteratedtriglycinesulfate detector.
- At least 24 h before, and during data acquisition, the spectrometer was continuously purged with dry air at a dew point of 40oC. Spectra of buffers and samples were acquired at 2 cm_1 resolution under the same scanning and temperature conditions. In the thermal denaturation experiments, the temperature was raised in 5oC steps from 20 to 95 °C.
- Before spectrum acquisition, samples were maintained at the desired temperature for the time necessary for the stabilization of temperature inside the cell (6 min).
- Spectra were collected and processed using the SPECTRUM software from Perkin-Elmer. Correct subtraction of H20 was judged to yield an approximately flat baseline at 1900-1400 cm_ 1, and subtraction of 2H20 was adjusted to the removal of the2H20 bending absorption close to 1220 cm_ 1
- ***Pavalaveerachunnam***is a mineral combination drug. FT-IR study was selected to identify the inorganic material of the test drug.The results are tabulated for further discussion.

INDUCTIVELY COUPLED PLASMA OPTICAL MASS SPECTROMETRY (ICP-MS)

Figure No :9 IMAGE OF ICP -MS

Inductively coupled plasma mass spectrometry (ICP-MS) is a type of mass spectrometry which is capable of detecting metals and several non-metals at concentrations as low as one part in 10^{15} (part per quadrillion, ppq) on non-interfered low-background isotopes. This is achieved by ionizing the sample with inductively coupled plasma and then using a mass spectrometer to separate and quantify those ions.

Compared to atomic absorption techniques, ICP-MS has greater speed, precision, and sensitivity. However, compared with other types of mass spectrometry, such as Thermal ionization mass spectrometry (TIMS) and Glow Discharge Mass Spectrometry (GD-MS), ICP-MS introduces many interfering species: argon from the plasma, component gases of air that leak through the cone orifices, and contamination from glassware and the cones. The variety of applications exceeds that of inductively coupled plasma atomic emission spectroscopy and includes isotopic speciation. Due to possible applications in nuclear technologies, ICP-MS hardware is a subject for special exporting regulations.

Inductively coupled plasma

An inductively coupled plasma is a plasma that is energized (ionized) by inductively heating the gas with an electromagnetic coil, and contains a sufficient concentration of ions and electrons to make the gas electrically conductive. Even a partially ionized gas in which as little as 1% of the particles are ionized can have the characteristics of a plasma (i.e., response to magnetic fields and high electrical conductivity). The plasmas used in spectrochemical analysis are essentially electrically neutral, with each positive charge on an ion balanced by a free electron. In these plasmas the positive ions are almost all singly charged and there are few negative ions, so there are nearly equal amounts of ions and electrons in each unit volume of plasma.

An inductively coupled plasma (ICP) for spectrometry is sustained in a torch that consists of three concentric tubes, usually made of quartz, although the inner tube (injector) can be sapphire if hydrofluoric acid is being used. The end of this torch is placed inside an induction coil supplied with a radio-frequency electric current. A flow of argon gas (usually 13 to 18 liters per minute) is introduced between the two outermost tubes of the torch and an electric spark is applied for a short time to introduce free electrons into the gas stream. These electrons interact with the radio-frequency magnetic field of the induction coil and are accelerated first in one direction, then the other, as the field changes at high frequency (usually 27.12 million cycles per second). The accelerated electrons collide with argon atoms, and sometimes a collision causes an argon atom to part with one of its electrons. The released electron is in turn accelerated by the rapidly changing magnetic field. The process continues until the rate of release of new electrons in collisions is balanced by the rate of recombination of electrons with argon ions (atoms that have lost an electron). This produces a 'fireball' that consists mostly of argon atoms with a rather small fraction of free electrons and argon ions. The temperature of the plasma is very high, of the order of 10,000 K. The plasma also produces ultraviolet light, so for safety should not be viewed directly.

The ICP can be retained in the quartz torch because the flow of gas between the two outermost tubes keeps the plasma away from the walls of the torch. A second flow of argon (around 1 liter per minute) is usually introduced between the central tube and the

intermediate tube to keep the plasma away from the end of the central tube. A third flow (again usually around 1 liter per minute) of gas is introduced into the central tube of the torch. This gas flow passes through the centre of the plasma, where it forms a channel that is cooler than the surrounding plasma but still much hotter than a chemical flame. Samples to be analyzed are introduced into this central channel, usually as a mist of liquid formed by passing the liquid sample into a nebulizer.

To maximise plasma temperature (and hence ionisation efficiency) and stability, the sample should be introduced through the central tube with as little liquid (solvent load) as possible, and with consistent droplet sizes. A nebuliser can be used for liquid samples, followed by a spray chamber to remove larger droplets, or a desolvatingnebuliser can be used to evaporate most of the solvent before it reaches the torch. Solid samples can also be introduced using laser ablation. The sample enters the central channel of the ICP, evaporates, molecules break apart, and then the constituent atoms ionise. At the temperatures prevailing in the plasma a significant proportion of the atoms of many chemical elements are ionized, each atom losing its most loosely bound electron to form a singly charged ion. The plasma temperature is selected to maximiseionisation efficiency for elements with a high first ionisation energy, while minimising second ionisation (double charging) for elements that have a low second ionisation energy.

Mass spectrometry

For coupling to mass spectrometry, the ions from the plasma are extracted through a series of cones into a mass spectrometer, usually a quadrupole. The ions are separated on the basis of their mass-to-charge ratio and a detector receives an ion signal proportional to the concentration.

The concentration of a sample can be determined through calibration with certified reference material such as single or multi-element reference standards. ICP-MS also lends itself to quantitative determinations through isotope dilution, a single point method based on an isotopically enriched standard.

Other mass analyzers coupled to ICP systems include double focusing magnetic-electrostatic sector systems with both single and multiple collector, as well as time of flight systems (both axial and orthogonal accelerators have been used).

Applications

One of the largest volume uses for ICP-MS is in the medical and forensic field, specifically, toxicology. A physician may order a metal assay for a number of reasons, such as suspicion of heavy metal poisoning, metabolic concerns, and even hepatological issues. Depending on the specific parameters unique to each patient's diagnostic plan, samples collected for analysis can range from whole blood, urine, plasma, serum, to even packed red blood cells. Another primary use for this instrument lies in the environmental field. Such applications include water testing for municipalities or private individuals all the way to soil, water and other material analysis for industrial purposes.

In recent years, industrial and biological monitoring has presented another major need for metal analysis via ICP-MS. Individuals working in plants where exposure to metals is likely and unavoidable, such as a battery factory, are required by their employer to have their blood or urine analyzed for metal toxicity on a regular basis. This monitoring has become a mandatory practice implemented by OSHA, in an effort to protect workers from their work environment and ensure proper rotation of work duties (i.e. rotating employees from a high exposure position to a low exposure position).

Regardless of the sample type, blood, water, etc., it is important that it be free of clots or other particulate matter, as even the smallest clot can disrupt sample flow and block or clog the sample tips within the spray chamber. Very high concentrations of salts, e.g. sodium chloride in sea water, can eventually lead to blockages as some of the ions reunite after leaving the torch and build up around the orifice of the skimmer cone. This can be avoided by diluting samples whenever high salt concentrations are suspected, though at a cost to detection limits.

ICP-MS is also used widely in the geochemistry for radiometric dating, in which it is used to analyze relative abundance of different isotopes, in particular uranium and lead. ICP-MS is more suitable for this application than the previously usedthermal ionization massspectrometry,asspecieswithhigh ionization energy such as osmium and tungsten can be easily ionized. For high precision ratio work, multiple collector instruments are normally used to reduce the effect noise on the calculated ratios.

In the field of flow cytometry, a new technique uses ICP-MS to replace the traditional fluorochromes. Briefly, instead of labelling antibodies (or other biological probes) with fluorochromes, each antibody is labelledwith a distinct combinations of lanthanides. When the sample of interest is analysed by ICP-MS in a specialised flow cytometer, each antibody can be identified and quantitated by virtue of a distinct ICP "footprint". In theory, hundreds of different biological probes can thus be analysed in an individual cell, at a rate of ca. 1,000 cells per second. Because elements are easily distinguished in ICP-MS, the problem of compensation in multiplex flow cytometry is effectively eliminated.

In the pharmaceutical industry, ICP-MS is used for detecting inorganic impurities in pharmaceuticals and their ingredients. New and reduced maximum permitted exposure levels of heavy metals form dietary supplements, introduced in USP (United States Pharmacopeia) <232>Elemental Impurities—Limits [1] and USP <233>Elemental Impurities—Procedures,[2] will increase the need for ICP-MS technology, where, previously, other analytic methods have been sufficient.

Elemental analysis

The ICP-MS allows determination of elements with atomic mass ranges 7 to 250 (Li to U), and sometimes higher. Some masses are prohibited such as 40 due to the abundance of argon in the sample. Other blocked regions may include mass 80 (due to the argon dimer), and mass 56 (due to ArO), the latter of which greatly hinders Fe analysis unless the instrumentation is fitted with a reaction chamber. Such interferences can be reduced by using a high resolution ICP-MS (HR-ICP-MS) which uses two or more slits constrict the beam and distinguish between nearby peaks. This comes at the cost of transmission, for example to distinguish Iron from Argon by take a resolving power of 10,000, which may reduce the Iron transmission by around 99%.

A single collector ICP-MS may use a multiplier in pulse counting mode to amplify very low signals, an attenuation grid or a multiplier in anologue mode to detect medium signals, and a Faraday cup/bucket to detect larger signals. A multi-collector ICP-MS may have more than one of any of these, normally Faraday buckets which are much less

expensive. With this combination, a dynamic range of 12 orders of magnitude, from 1 ppq to 100 ppm is possible.

ICP-MS is a method of choice for the determination of cadmium in biological samples.[4] Unlike atomic absorption spectroscopy, which can only measure a single element at a time, ICP-MS has the capability to scan for all elements simultaneously. This allows rapid sample processing. A simultaneous ICP-MS that can record the entire analytical spectrum from lithium to uranium in every analysis won the Silver Award at the 2010 Pittcon Editors' Awards. An ICP-MS may use multiple scan modes, each one striking a different balance between speed and precision. Using the magnet alone to scan is slow, due to hysteresis, but is precise. Electrostatic plates can be used in addition to the magnet to increase the speed, and this, combined with multiple collectors, can allow a scan of every element from Lithium 6 to Uranium Oxide 256 in less than a quarter of a second. For low detection limits, interfering species and high precision, the counting time can increase substantially. The rapid scanning, large dynamic range and large mass range is ideally suited to measuring multiple unknown concentrations and isotope ratios in samples that have had minimal preparation (an advantage over TIMS), for example seawater, urine, and digested whole rock samples. It also lends well to laser ablated rock samples, where the scanning rate is so quick that a real time plot of any number of isotopes is possible.This also allows easy spatial mapping of mineral grains.

Sample introduction

The first step in analysis is the introduction of the sample. This has been achieved in ICP-MS through a variety of means.

The most common method is the use of *analytical nebulizers.* Nebulizer converts liquids into an aerosol, and that aerosol can then be swept into the plasma to create the ions. Nebulizers work best with simple liquid samples (i.e. solutions). However, there have been instances of their use with more complex materials like a slurry. Many varieties of nebulizers have been coupled to ICP-MS, including pneumatic, cross-flow, Babington, ultrasonic, and desolvating types. The aerosol generated is often treated to limit it to only smallest droplets, commonly by means of a Peltier cooled double pass or cyclonic spray chamber. Use of autosamplers makes this easier and faster, especially for routine work

and large numbers of samples. A DesolvatingNebuliser (DSN) may also be used; this uses a long heated capillary, coated with a fluoropolymer membrane, to remove most of the solvent and reduce the load on the plasma. Matrix removal introduction systems are sometimes used for samples, such as seawater, where the species of interest are at trace levels, and are surrounded by much more abundant contaminants.

Laser ablation is another method. While being less common in the past, is rapidly becoming popular has been used as a means of sample introduction, thanks to increased ICP-MS scanning speeds. In this method, a pulsed UV laser is focused on the sample and creates a plume of ablated material which can be swept into the plasma. This allows geochemists to spacially map the isotope composition in cross-sections of rock samples, a tool which is lost if the rock is digested and introduced as a liquid sample. Lasers for this task are built to have highly controllable power outputs and uniform radial power distributions, to produce craters which are flat bottomed and of a chosen diameter and depth.

For both Laser Ablation and DesolvatingNebulisers, a small flow of Nitrogen may also be introduced into the Argon flow. Nitrogen exists as a dimer, so has more vibrational modes and is more efficient at receiving energy from the RF coil around the torch.

Other methods of sample introduction are also utilized. Electrothermal vaporization (ETV) and in torch vaporization (ITV) use hot surfaces (graphite or metal, generally) to vaporize samples for introduction. These can use very small amounts of liquids, solids, or slurries. Other methods like vapor generation are also known.

Plasma torch

The plasma used in an ICP-MS is made by partially ionizing argon gas (Ar ’! Ar^+ + e^-). The energy required for this reaction is obtained by pulsing an alternating electric current in wires that surround the argon gas.

After the sample is injected, the plasma’s extreme temperature causes the sample to separate into individual atoms (atomization). Next, the plasma ionizes these atoms (M ’! M^+ + e^-) so that they can be detected by the mass spectrometer.

An inductively coupled plasma (ICP) for spectrometry is sustained in a torch that consists of three concentric tubes, usually made of quartz. The two major designs are the

Fassel and Greenfield torches.The end of this torch is placed inside an induction coil supplied with a radio-frequency electric current. A flow of argon gas (usually 14 to 18 liters per minute) is introduced between the two outermost tubes of the torch and an electrical spark is applied for a short time to introduce free electrons into the gas stream. These electrons interact with the radio-frequency magnetic field of the induction coil and are accelerated first in one direction, then the other, as the field changes at high frequency (usually 27.12 MHz). The accelerated electrons collide with argon atoms, and sometimes a collision causes an argon atom to part with one of its electrons. The released electron is in turn accelerated by the rapidly changing magnetic field. The process continues until the rate of release of new electrons in collisions is balanced by the rate of recombination of electrons with argon ions (atoms that have lost an electron). This produces a 'fireball' that consists mostly of argon atoms with a rather small fraction of free electrons and argon ions.

Advantage of argon

Making the plasma from argon, instead of other gases, has several advantages. First, argon is abundant (in the atmosphere, as a result of the radioactive decay of potassium) and therefore cheaper than other noble gases. Argon also has a higher first ionization potential than all other elements except He, F, and Ne. Because of this high ionization energy, the reaction ($Ar^+ + e^-$ '! Ar) is less energetically favorable than the reaction ($M^+ + e^-$ '! M). This ensures that the sample remains ionized (as M^+) so that the mass spectrometer can detect it.

Argon can be purchased for use with the ICP-MS in either a refrigerated liquid or a gas form. However it is important to note that whichever form of argon purchased, it should have a guaranteed purity of 99.9% Argon at a minimum. It is important to determine which type of argon will be best suited for the specific situation. Liquid argon is typically cheaper and can be stored in a greater quantity as opposed to the gas form, which is more expensive and takes up more tank space. If the instrument is in an environment where it gets infrequent use, then buying argon in the gas state will be most appropriate as it will be more than enough to suit smaller run times and gas in the cylinder will remain stable for longer periods of time, whereas liquid argon will suffer loss to the environment due to venting of the tank when stored over extended time frames. However if the ICP-MS is to

be used routinely and is on and running for eight or more hours each day for several days a week, then going with liquid argon will be the most suitable. If there are to be multiple ICP-MS instruments running for long periods of time, then it will most likely be beneficial for the laboratory to install a bulk or micro bulk argon tank which will be maintained by a gas supply company, thus eliminating the need to change out tanks frequently as well as minimizing loss of argon that is left over in each used tank as well as down time for tank changeover.

There are rare ICP-MS solutions that utilize helium for plasma generation.

Transfer of ions into vacuum

The carrier gas is sent through the central channel and into the very hot plasma. The sample is then exposed to radio frequency which converts the gas into a plasma. The high temperature of the plasma is sufficient to cause a very large portion of the sample to form ions. This fraction of ionization can approach 100% for some elements (e.g. sodium), but this is dependent on the ionization potential. A fraction of the formed ions passes through a ~1 mm hole (sampler cone) and then a ~0.4 mm hole (skimmer cone). The purpose of which is to allow a vacuum that is required by the mass spectrometer.

The vacuum is created and maintained by a series of pumps. The first stage is usually based on a roughing pump, most commonly a standard rotary vane pump. This removes most of the gas and typically reaches a pressure of around 133 Pa. Later stages have their vacuum generated by more powerful vacuum systems, most often turbomolecular pumps. Older instruments may have used oil diffusion pumps for high vacuum regions.

Sample preparation

For most clinical methods using ICP-MS, there is a relatively simple and quick sample prep process. The main component to the sample is an internal standard, which also serves as the diluent. This internal standard consists primarily of deionized water, with nitric or hydrochloric acid, and Indium and/or Gallium. Depending on the sample type, usually 5 ml of the internal standard is added to a test tube along with 10–500 microliters of sample. This mixture is then vortexed for several seconds or until mixed well and then loaded onto the autosampler tray. For other applications that may involve very viscous samples or samples that have particulate matter, a process known as sample digestion may have to be carried out, before it can be pipetted and analyzed. This adds an extra first step to the above process, and therefore makes the sample prep more lengthy.

4.3.PRECLINICAL TOXICOLOGICAL STUDY OF *PAVALA VEERACHUNNAM* ON RAT

ACUTE ORAL TOXICITY STUDY (LD50 Determination)

Experimental Animals

Adult albino wistar rats of either sex weighing around 125-180gms were used. The animals were maintained at normal room temperature with a humidity of 55 +5%. All the animals were fed with pellet diet obtained from Poultry Research Station, Nandanam, Chennai - 35 and tap water *ad libitum* throughout the experimental period. The animals were acclimatized to the laboratory conditions before experimental procedures were started. The experimental protocol for the ***Pavalaveerachunnam*** , were approved by the college of Annai Velankanni Pharmacy college,Chennai.

For carrying out oral toxicity study **Organization for economic cooperation andDevelopment (OECD)** guidelines 423 were followed. It is a stepwise procedure with three animals of a single sex per step. Depending on the mortality and/or morbidity of the animals a few steps may be necessary to judge the toxicity of the test substance. This procedure has advantage over other methods because of minimal usage of animals while allowing for acceptable data. The method uses defined doses (5, 50, 300, 2000mg/kg body weight) and the results allow a substance to be ranked and classified according to the globally harmonized system. The starting dose of ***Pavalaveerachunnam*** was 2000mg/kg bodyweight p.o. The dose was administered to the rats which were fasted overnight with water *ad libitum* and observed for signs of toxicity. The same dose was once again tried with another three rats and were observed for 72 hours for symptoms like change in skin color, salivation, diarrhea, sleep, tremors, convulsions and also respiratory, autonomic and CNS effects.

SUB ACUTE TOXICITY STUDY (OECD GUIDELINES 423)

Humans are more often exposed to chemicals at levels much lower than those that are actually fatal, but they are exposed over longer periods of time. To assess the nature of the toxic effects under these more realistic situations, short term and long term toxicity

studies are conducted. Equal number of males and females should be used. Generally at least 6 rats are used in each dose group as well as control group.

Route of administration

The test drug (***Pavalaveerachunnam***) should be administered by the route of the intended use or exposure in humans. For most chemicals the most preferred route is the oral route. The preferred procedure is to incorporate the chemical in the diet, although the drinking water is sometimes used as a vehicle. The latter method is advisable when the chemical may react with a component in the diet. The chemical may be administered by oral gavage needle.

Dosage and duration

Since the aim of these studies is to determine the nature and site of the toxic effects as well as the "no-effect level", it is advisable to select three doses. These doses are generally selected on the basis of the information obtained in the acute toxicity studies.

Observations and examinations

Body weight and food consumption

These should be determined weekly. Decreased body weight gain is simple yet sensitive index of toxic effects. Food consumption is also a useful indicator. In addition a marked decrease in the consumption can induce effects that mimic or aggravate the toxic manifestations of the chemical.

General observations

These should include appearance, behavior, and any abnormality. Dead and Moribund animals should be removed from the cages for gross and possibly for microscopic examination. Frequent observation is necessary to minimize cannibalism.

Laboratory tests

Hematologic examinations generally include hematocrit, hemoglobin, erythrocytecount, total leukocyte count, and differential leukocytes count. Clinical laboratory tests usually include fasting blood glucose, Aspartate aminotransferase (AST), Alanine aminotransferase (ALT) alkaline phosphatase (ALP) , total Bilirubin (tb), blood

urea creatinine, uric acid and other elements such as sodium, potassium, calcium, and phosphorous.

Procedure

Adult healthy albino rats of either sex weighing 125-180 g were selected for the study. The rats were divided into Three groups of six animals each. The drug was administered in doses of 200 and 400 mg/kg daily once in the morning for 28days. Group I received the vehicle (Normal Saline) once daily and served as solvent control. Group II and III received***Pavalaveerachunnam***with Honey once daily in a dose of 200 and 400 mg/kg respectively. The animals were placed in cages and the body weight and feed consumption were recorded frequently. Simultaneously observation was made for any toxic symptoms or mortality.

On 29th day animals were anesthetized by intra peritoneal (i.p) administration of thiopental sodium and blood was collected by retro-orbital puncture and the serum was separated. Hematological parameters like RBC, WBC and Hb were estimated from blood. The serum was subjected to biochemical analysis like aspartate aminotransferase (AST), alanine amino transferase (ALT), alkaline phosphatase, (ALP) total bilirubin, urea, uric acid and creatinine. Vital organs like liver, Brain, Heart, lungs and kidneys, were dissected out carefully; extra tissues and blood were removed, observed for pathological changes and weighed. It was kept in 10% formalin and subjected for histopathological analysis.

Instruments used

Auto analyzer

Semi auto analyzer ERBA, was used to estimate biochemical parameters viz. Aspartate Aminotransferase (AST), Alanine Aminotransferase (ALT), Alkaline Phosphates (ALP), Total Bilirubin (TB), Urea, creatinine, uric acid.

Hematological studies

The following hematological parameters were estimated by standard procedures.

Estimation of Total R.B.C. count (Ghai, C.L. (1993).

The enumeration of red blood corpuscles was carried out on blood samples using haemocytometer as per standard methods. The blood was taken in the R.B.C. pipette up to 0.5 marks and diluted with R.B.C. diluting fluid (Hyme's fluid) up to 101 marks. It was mixed well and mounted on Neubauer's counting chamber. The red blood cells were then counted using a microscope and their number in undiluted blood was calculated. R.B.C. count in blood is expressed as cells in millions/mm3.

Estimation of Total W.B.C. Count (Ghai. 1993).

The blood was taken in W.B.C. pipette up to 0.5 mark and diluted with W.B.C. diluting fluid (1-2% acetic acid stained lightly with gentian violet), where acetic acid destroy the red blood corpuscles and gentian violet stains the nuclei of white blood cells. The fluid in the pipette was mixed well and mounted in the counting chamber. The W.B.C. count was then counted and their number in undiluted blood was calculated. W.B.C. count in blood is expressed as cells in 1000/mm3

Estimation of Hemoglobin (Hb) concentration (Ghai. 1993).

Haemoglobin in the red blood corpuscles was converted to acid haematin by adding N/10 hydrochloric acid. The brown colour developed was matched against standard brown tinted glass in the comparator by direct vision. The haemoglobin concentration in blood is expressed as grams per 100 ml of blood (g%).

Serum Analysis

The serum was analysed for the following parameters.

a. Aspartate Aminotransferase (AST)

b. Alanine Aminotransferase (ALT)

c. Alkaline Phosphatase (ALP)

d. Total Bilirubin (TB)

e. Urea

f. Creatinine

g. Uric Acid

The separated serum was subjected to biochemical analysis by ERBA diagnostic kit for the following parameters like urea, uric acid, creatinine, total bilirubin AST, ALT, ALP.

Estimation of urea

Estimation of urea was carried out using ERBA kit by the urease method.

Urea Reagent

A-ketoglutarate	75 mmol/L
NADH	0.32 mmol/L
Urease	> 8.00 IU/L
GLDH	> 1.00 IU/L
ADP	1.2 mmol/L
aTris Buffer pH 7.9 ± 0.1 at 25°C	100 mmol/L

Urea standard

Urea	50mg/dl

Assay Procedure

Two test tubes were marked as standard and test. 1000 L of working reagent was pipetted into both the test tubes. 20 L of standard urea was added to the standard test tube. 20 L of serum was added to the other test tube. A blank was also prepared with distilled water. It was then mixed well and the absorbant was monitored at 340 nm. The change in absorbance for the standard and the test were calculated using the formula

$$\Delta A = A_1 - A_2$$

$$\text{Urea} = \frac{\Delta A \text{ of test}}{\Delta A \text{ of standard}} \times \text{Concentration of Standard (mg / dl)}$$

Uric acid

Uric acid was estimated using modified Trinder method using ERBA diagnostic kit.

Uric acid reagent

$$\text{Uric acid (mg/dl)} = \frac{\text{Abs.of test}}{\text{Abs. of standard}} \times \text{Concentration of Standard(mg /dl)}$$

4-aminoantipyrine	0.5 mmol/L
TBBH	1.75 mmol/L
Uricase	120 IU/L
Peroxidase	500 IU/L
Tris buffer pH 8.25 ± 0.1 at 20°C	50 mmol/L
Uric acid standard 6mg/dl	(0.36 mmol/L)

Assay Procedure

Three test tubes were marked as blank, standard and test. 1000 µL of working reagent was added to all the three test tubes. 20 µL of distilled water was added to the blank. 20 µL of standard uric acid solution was added to the standard test tube. To the test 20 µL of serum was added. It was then mixed well and incubated for 5 mins at 37° C. The absorbance of standard and each test were read at 505 nm against a reagent blank.

Creatinine

Creatinine was estimated by Jaffe's method using ERBA diagnostic kit.

Reagent 1: picric acid reagent

Picric acid 25.8 mmol/L

Reagent 2: sodium hydroxide reagent

Sodium hydroxoide95 mmol/L

Creatinine standard

Creatinine - 2mg/dl (0.166 mmol /L). Equal Volumes of reagent 1 and 2 were mixed and waited for 15 minutes before use.

Procedure

Two test tubes were marked as standard and test. 1000 µ L of working reagent was added to both the test tubes. To the standard test tube 100 µ L ofcreatinine and to the test 100 µ L of serum were added. It was then mixed well and the initial absorbance (Al) was read immediately. After 80 sees the final absorbance (A2) was read after mixing at 505 nm. $\Delta A = A_2 - A_1$

$$\text{Creatinine} = \frac{\Delta A \text{ of Test}}{\Delta A \text{ of Standard}} \times \text{Concentration of Standard (mg}$$

Liver function tests

Bilirubin (Diazo method)

Total biliriubin was estimated by diazo method using ERBA diagnostic kit.

Principle

Biliriubin reacts with diazotized sulphanilic acid in acidic medium to form pink coloredazobilirubin with absorbance directly proportional to bilirubin concentration.

Reagent composition

Reagent 1: Total biliriubin reagent

Surfactant	1.000%
Hcl	100 mmol/L
Sulphanilic acid	5 mmol/L

Reagent 2: Direct biliriubin reagent

Sulphanilic acid	10 mmol/L
Hcl	100 mmol/L

Reagent 3: Sodium nitrite reagent

Sodium nitrite 144 mmol/L

Reagent preparation

Total biliriubin (vol 25 ml) = 25 ml reagent 1 + 0.5 ml reagent 3

Assay procedure

3 test tubes were taken and labelled as blank, standard and test. 500 µl of working reagent was added to all the three test tubes. 25 µl of distilled water was added to blank, 25 µl of standard solution was added to the standard and 25 µl of serum was added to the test. It was mixed well and incubated for 5mins at 37°C. The absorbance was read at 546 nm against reagent blank.

T. biliriubin (mg/dl) = (Absorbance of test/ Absorbance of standard) x Conc of std(mg/dl)

Aspartate aminotransferase (AST)

AST was estimated by International Federation Of Clinical Chemistry (IFCC) method using ERBA diagnostic kit. AST occurs in all human tissues like liver, kidney, heart and skeletal muscle.

Reagent composition

Reagent 1: AST reagent

2- oxoglutarate	12 mmol/L
L- aspartate	200 mmolL
MDH	545 U/L
LDH	909 U/L
NADH	0.18 mmol/L
Tris buffer	pH 7.8 80 mmol/L
EDTA	5 mmol/L

Assay procedure

1000 µ l of working reagent after reconstitution was added to a test tube and 100 µ l of serum was added to it, mixed well and aspirated. The absorbance was read at 340 nm. The absorbance change was converted to international units using the formula

$$IU/L = \frac{\Delta A/min) \text{ x total reaction volume x } 10^3}{\text{Sample volume absorptivity x P}}$$

Alanine aminotransferase (ALT)

ALT was estimated by International Federation Of Clinical Chemistry (IFCC) method using ERBA diagnostic kit.

Reagent composition

Reagent: ALT reagent

L- alanine	500 mnol/L
NADH (yeast)	0.18 mmol/L
LDH	>1820 IU/L

2-oxoglutarate 12 mmol/L

Tris buffer pH 7.5 80 mmol/L

Assay procedure

1000 μ l of working reagent after reconstitution was added to a test tube and 100 μ l of serum was added to it and mixed well and aspirated. The absorbance was read at 340 nm. International units (IU) of activity was calculated using the formula

$$IU/L = \frac{\Delta A/min) \times \text{total reaction volume} \times 10^3}{\text{Sample volume} \times \text{absorptivity} \times P}$$

Alkaline phosphatase

Alkaline phosphatase was estimated by p-Nitrophenyl phosphate (pNPP kinetic method)

Principle

ALP at an alkaline pH, hydrolyses P-nitrophenylphosphate to form P-nitrophenoland phosphate. The rate of formation of p-nitro phenol is measured as an increase in absorbance which is proportional to the ALP activity in the sample.

Working reagent 1: substrate tablet was dissolved in 3.2 ml of buffer reagent. (10x3 ml pack) 1 ml of working reagent was pipetted out in to a clean dry test tube and 0.02 ml of serum was added, mixed well and initial absorbance Ao was observed after lmin. The absorbance reading was repeated every 1, 2 and 3 min. The mean absorbance change per min(Δ A /min) was calculated using the formula *ALP activity inU/ L* $=\Delta A$/min-2754

Histopathology

Liver, lungs, spleen and kidneys were evaluated for histopathological changes. After collection of organs, the tissues were stored in 10% formalin for 48 hrs. The solution was changed after 24 his. In the case of kidneys, two incomplete parallel cuts were made from cortex to pelvis and one longitudinal cut was made which divides the kidney into two halves. In the case of liver one piece from each lobe was used. The tissues were then wax embedded. The tissues were then wax embedded as below:

Solvent	Duration
50% Alcohol	Holding Period
70% Alcohol	30 minutes
95% Alcohol	20 minutes
100% Alcohol	30 minutes
Chloroform	30 minutes
Wax	10 minutes

They were then blocked in wax and allowed to set on a cold plate. One cooled they were cut into 5u size sections. The sections were kept in slides and stained with haematoxyline and eosin.

4.4 PHARMACOLOGICAL STUDY

HEPATOPROTECTIVE ACTIVITY OF *PAVALAVEERA CHUNNAM*

PARACETAMOL- INDUCED LIVER DAMAGE IN RATS PROCEDURE

Paracetamol is one of the most commonly and widely used analgesic, anti-pyretic drug, which shows a high margin of safety. However, when used in high doses it can lead to hepatic damage, It gets metabolized to an active metabolite N-acetyl-p-benzoquinone imine by the cytochrome-p-450 microsomal enzyme system,which results in an oxidative stress producing liver glutathione and glycogen depletion and hepatic.

Methodology

Wister rats of either sex weighing 150-200g are used. Paracetamol 2g/kg body weight is administered orally as a single dose. The animals are given the test drug for 6 days prior to Paracetamol administration and on the seventh day along with paracetamol. The animal are sacrificed after 24 hrs and the blood /serum is used for biochemical analysis and the liver for histopathological studies

HYPOLIPIDEMIC ACTIVITY OF *PAVALAVEERA CHUNNAM*

Cholesterol- Diet induced Hyperlipedemia in mice

Hyperlipidemia is the one of the major risk factor for the development of coronary heart disease. Currently available hyperlipidemic drugs have been associated with number of side effects. A number of herbal medicines are used for controlling hyperlipidemia because of the undesirable side effects and contraindications of synthetic drug

Methodology

Swiss albino Mice of either sex weighing 20-3Ogm are fed on a cholesterol diet consisting of cholesterol (l%),Sodium cholate(0.5%), sucrose(30%), cascien(10%), butter(5%) and standard chow (53.5%) for seven days. The animals are divided in 5 groups of control, test drug and standard treated animals. Group I Received Normal saline and served as control. Group II - positive control (400mg/kg.p.o)Cholesterol induced diet, Group III - Group II + Atorvastatin (13mg/kg.p.o),Group IV - Group II + Sample drug 200mg/kg bw, Group V- Group II +Sample drug 400mg/kg bw, The study is considered in two stages, in the preliminary stage effective hypolipedemic doses of test and standard drugs are worked out and in the final stage the effect of test and standard drugs are studied in three graded doses depending upon the results of preliminary stage and the LD 50 of test compound. Finally the LD test and standard drugs are calculated to assess relative potency of the test. The lipid profile includes total cholesterol, LDL, HDL, Triglycerides.

DIURETIC ACTIVITY OF PAVALAVEERA CHUNNAM(LIPSCHITZ TEST)

PROCEDURE

Healthy adult Wister male rats of weighing 150-200 g procured from the animal house of - College of Pharmacy were used for the study. The animals were maintained in well ventilated room temperature with natural 12 + 12 day and night cycle in the polypropylene cages. The animals were fed with balanced diet that is standard rodent pellet diet (Hindustan Lever Ltd.) and water ad libitum.

The animals were housed for 1 week prior to the experiment to acclimatize to the laboratory conditions. Approval for the research work and ethical clearance was obtained from the College of Pharmacy, (Ethical Committee No SBCP / 2015-2016/ CPCSEA). Wistar rats were divided into 4 groups (6 each). The animals of group (I) served as normal control (Vehicle) which received normal saline water (2 ml/kg b.w., orally) only. The animals of group (II) served as standard control which received frusemide (5mg/kg b.w., orally). Groups (III) received sample drug 200mg/kg bw. Group(IV) received sample drug 400mg/kg bw-orally. The method is based on water and sodium excretion in test animals as compared to rats treated with high dose of urea. The method of Lipschitz et al. was employed for the assessment of diuretic activity. Male Wistar rats weighing 150 to 200 g were used. They were placed in metabolic cages provided with a wire mesh bottom and a funnel for collecting the urine. Stainless steel sieves were placed in the funnel to retain the feces, allowing only urine to flow down for collection and measurement. The food and water are withdrawn 15 h prior to the test. Three animals were placed in one metabolic cage. The rats of each group were treated with drugs as per the details mentioned above. Additionally 5 ml of normal saline solution per 100 g was administered orally to all rats. Urine excretion was recorded after 15mts,30mts,lhr,2hr,3hr and 4hr. The sodium and potassium contents of the collected urine were estimated by Flame Photometer (Toshniwal group model TCM-35). The instrument was calibrated with standard solutions containing different concentrations of Na+ and K+. The conductivity was directly determined on fresh urine samples using a conductometer (Toshniwal group model TCM-15). PH was measured with a digital pH meter (MK-VI, Unique instruments & machineries, Calcutta) on fresh urine sample.

LITHOTRIPTIC ACTIVITY OF *PAVALAVEERA CHUNNAM*

Experimental animals

Adult male albino rats of Wistar strains, weighing between 150 – 250g, obtained from Small Animal Breeding Center, were used for the experimental studies. The animals were acclimatized to the animal house condition for ten days. The animals were fed with commercial rat field and water was given ad libitum.

Experimental induction of calcium oxalate lithiasis in rats

The method adopted was that of Chow et al. (1975).

Preparation of calculi producing diet (CPD)

The method adopted was that of Chow et al. (1975). Commercial rat feed was finely powdered, supplemented with sodium glycollate (3 % concentration, w/w) and pelleted with water. The pellets were dried in an oven at 30°C for 2 days and used as the calculi producing diet.

Experimental setup

The animals were divided into 4 groups comprising of six animals in each. Group I Received Normal saline and served as control. Group 11- Negative control (70mg/ kg.p.o)CPD , Group III – Cystone (500mg/kg.p.o) Group IV - Sample drug 200mg/kg bw, Group V- Sample drug 400mg/kg bw, Rats fed with CPD for 30 days to induce stone formation. After the experimental period, 24 h urine samples were collected using hydrochloric acid as preservative. The animals were sacrificed by decapitation, liver and kidneys were excised from the body and their weights were *recorded, a small portion* of both kidney and Jiver tissues were preserved for histo pathological and electron microscopic studies

4.5.ANTI-MICROBIAL ACTIVITIESBY KIRBY BAUER METHOD

Aim:

The antimicrobial activity of ***Pavalaveerachunnam*** was adapted through Kirby-Bauer method(Agar diffusion testing).

Components of Muller Hinton agar medium:

Beef extract - 2gm/lit

Acid Hydrolysate of Casein - 17.5 gm/lit

Starch - 1.5 gm/lit

Agar – 17gm/lit

Distilled water - 1000 ml

PH - 7.3± 0.1 at 25^0C

Procedure:

The sterilized (autoclaved at 120^0C for 30 min) medium (40-50^0C) was inoculated (1 ml/100 ml of medium) with the suspension (150 cells per ml) of the Micro-organism (matched to McFarland turbidity standard) and poured in to a Petri dish to give depth of 3-4 mm. The paper saturated with the test compounds - ***Pavalaveerachunnam***was placed on the solidified medium.The plates were pre incubated for 1 hour at room temperature and incubated at 37^0C for 24 and 48 hours for anti-bacterial activities respectively. Amikacin is used as standard for anti-bacterial and Ketokonazole is used as standard for anti-fungal respectively at the concentration of 50 mcg / disc standard for an In-vitro antimicrobial activity of ***Pavalaveerachunnam*** was screened against bacteria strains such as *Klebsiellaaerogenes Staphylococcus pyogenes, Escherichia coli, Staphylococcus albus, Salmonella typhi, Proteus vulgaris, p.aeruginosa* and fungal strain *Candida albicans.*

The prepared disc of***Pavalaveerachunnam*** are placed over the incubated plate using sterile forceps and incubated for 24 hours at 37^0C. The plates after 24 hours incubation are observed for the zone of inhibition.

5. RESULT AND DISCUSSION

The Siddha mineral animal drug *Pavalaveera Chunnam* had been subjected to various studies to establish the works of *Siddhars* to be true. Literary collections, physicochemical and Elemental analysis, toxicological study, pharmacological study and clinical study are done to prove the activity of *Pavalaveera Chunnam in Hepatoprotective,Hypolipidemic,Diuretic and Lithotriptic* activities.

The experimental analysis was done to standardize the *Pavalaveera Chunnam* by its chemical compounds and particle size.

***SIDDHA* STANDARDIZATION METHODS:**

Siddhars used these following standardization methods to ensure the safety and efficacy of the Chunnam. It shows the effectiveness of the drug.

Table No:4 Results of Siddha standardization

S.No	Parameter	Results of VP	Interpretation
1.	Colour no shining	Mild grey colour, process	Indicates complete calcination
2.	Odour	Odourless process	Indicates complete calcination
3.	Taste	Tasteless	Indicates complete calcinations process
4.	Finger Print Test furrow of fingers	Impinged in the powder.	Indicates fine particles of
5.	Floating on Water	Floats on water	Lightness of drug.
6.	Lustre	Lustreless	No glowing particles seen.lt indicates complete calcinations process

- The test drug PVC being .tasteless, odourless, lusterless and irreversible on heating, indicates complete calcinations process.
- It is an ideal colour for ***Chunnam.*** Without shining indicates no free form of metals present in that drug.

- The test drug which was float on water has less specific gravity, possesses specific gravity less than the water.
- On Finger print test, Only the particles which are in micro fine size that can enter into the furrows of the finger print.
- PVC getting impinged in the furrows of fingerprint, indicates presence of microfine particles and complete calcinations process

AS PER MODERN STANDARDIZATION METHODS

Table No 5:Organoleptic characters of *Pavalaveerachunnam*

S.NO	Parameter	Result
1.	Colour in daylight	White colour
2.	Appearance	Powder
3.	Sense on touch	Nice
4.	Sense on smell	Odorless
5.	Sense on taste	Taste less
6.	Solubility	Sparingly soluble in water and well soluble in acids (con.HCl and con.H_2SO_4)
7.	Action on heat	No change
8.	Flame test	No change

Action on heat:

No strong white furmes evolved indicating the absence of carbonate

Flame test:

No bluish green flame appeared indicating absence of copper.

4.2.2.1 PHYSICO-CHEMICAL ANALYSIS:

1. Determination of loss on drying (LOD)(Normal value -less than 8%)

Loss in weight on drying = 0%

The stability of a drug and its shelf-life are reliant to moisture content of that drug. Determination of moisture (loss on drying) in a drug is one of the important tests in pharmaceutical analysis (kasture A.V, 2008).

'Physico-chemical analysis of ***Pavalaveerachunnam*** showed that loss on drying (LOD) is 0 % which shows that no moisture content present in the prepared medicine. Increased moisture content is the issue for instability of drug and lesser shelf-life of a drug. Since, ***Pavalaveerachunnam***has been well prepared it could get maximum stability and better shelf-life. Longer shelf-life for***Chenduram***mentioned in siddha literature is thus justified from the above observation.

2. Determination of ash values:

A. Total ash content=3.9%(Normal value - less than 80%)

The ash limit tests are designed to measure the amount of residual substances when a sample is ignited under the conditions specified in the individual monograph. A high ash value is indicative of contamination, substitution, adulteration, or carelessness in preparing the drug. The total ash values of ***Pavalaveerachunnam***were 3.9%, the value of total ash in the formulation is comparatively low. The value of total ash indicates that the inorganic contents of the formulation are below the limits. It signifies the ash value determination as an important parameter to standardize the herbal drugs.

B. Acid insoluble ash=0.8% (Normal value -less than 60%)

The acid-insoluble ash limit test is designed to measure the amount of ash insoluble to diluted hydrochloric acid. Acid-insoluble ash value of the prepared formulation (0.8%) shows that a very small amount of the inorganic component is insoluble in acid. It indicates that adulteration of raw ingredients by substances, such as silica and husk, is very less, and a low acid-insoluble ash value may also affect the amount of the component absorbed in the gastro-intestinal canal when taken orally.

The test drug **Pavalaveera chunnam**having, lower the acid insoluble value better will be the drug quality.

3. Determination of Alcohol-soluble and water-soluble extractive values

Alcohol soluble and water soluble extractive values of **Pavalaveerachunnam** are depicted in Table .5 which shows 43.3% of alcohol-soluble extractive value and 14.2% of water-soluble extractive value of the formulation. Lesser water-soluble extractive value

implies that alcohol is a better solvent of extraction for the formulation than water. The results of Alcoholic and water soluble extracts of the formulation show that mineral contents of the formulations are more soluble in alcohol than water and a lesser water soluble extractive value (14.2%) of the formulation. Hence honey should be used as adjuvant for this preparation.

Microbial Limit Tests:

Table No :6 Results for Microbial limit test on *Pavalaveerachunnam*:

S.No	Microbes	Colony measurements	Normal limits
1.	Total viable aerobic count	1.0 $x10^4$ col/g	1 $x10^5$ col/g
2.	Total *Enterobacteriaceae*	Nil	1 $x10^3$ col/g
3.	Total fungal count	1.0 $x10^4$ col/g	1 $x10^4$ col/g
	Test for specific pathogen		
1.	*Salmonella sp*	Nil	Nil
2.	*Staphylococcus aureas*	Nil	Nil
3.	*E.coli*	Nil	Nil
4.	*Pseudomonas aeruginosa*	Nil	Nil

- Total viable aerobic counts within the normal level.
- Total *Enterobacteriaceae* counts within the normal level.
- Total fungal count within the normal level.
- Specific pathogens like *Salmonella sp., Staphylococcus aureus, E.coli* and *Pseudomonas aeruginosa*are Nil.

Hence, the test drug is free from any microbial contamination and it has standard quality.

THIN LAYER CHROMATOGRAPHY:

Thin-layer chromatography (TLC) is a chromatographic technique that is useful for separating organic compounds. Because of the simplicity and rapidity of TLC, it is often used to monitor the progress of organic reactions and to check the purity of products. TLC is a simple, quick, and inexpensive procedure that gives how many components are

in a mixture. TLC is also used to support the identity of a compound in a mixture when the Rf of a compound is compared with the Rf of a known compound (preferably both run on the same TLC plate).Chromatography works on the principle that different compounds will have different solubility and absorption to the two phases between which they are to be partitioned. As the solvent rises by capillary action up through the adsorbent, differential partitioning occurs between components of the mixture dissolved in the solvent the stationary adsorbent phase. The more strongly a given component of a mixture is adsorbed onto the stationary phase, the less time it will spend in the mobile phase and the more slowly it will migrate up the plate.

TLC SHEET OF *PAVALAVEERA CHUNNAM*

Under UV 254mm and 366 mm

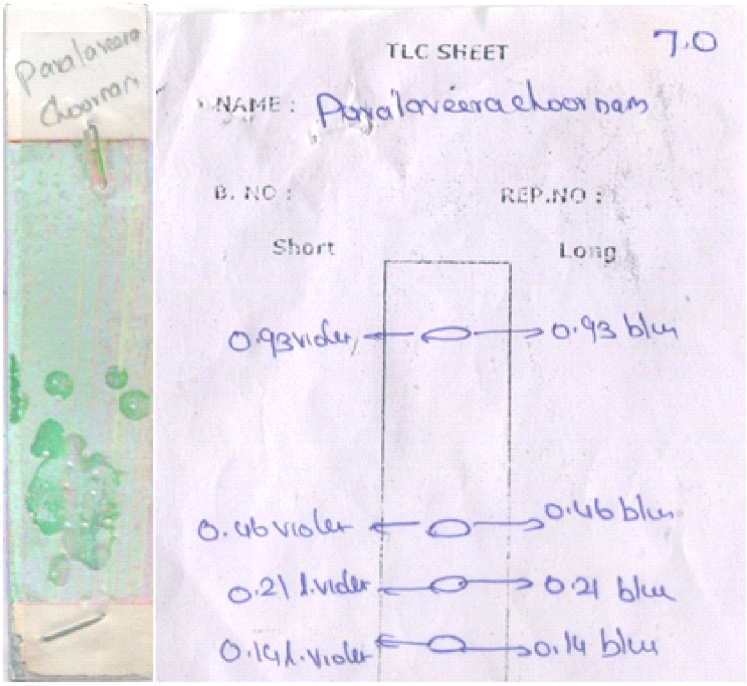


Figure :11 TLC SHEET OF PAVALAVEERA CHUNNAM

Interpretation

Under UV 254mm and 366mm TLC result shows RF value (0.93blue) indicates the presents of various chemical compounds in this drug.

CHEMICAL ANALYSIS:

Table No:7 Preliminary Basic and Acidic Radical Studies

Sl.No	PARAMETERS	RESULT
1.	Calcium	**Present**
2.	Iron (ferric)	Absent
3.	Iron (ferrous)	**Present**
4.	Zinc	Absent
5.	Sulphate	**Present**
6.	Chloride	**Present**
7.	Phosphate	Absent
8.	Carbonate	Absent
9.	Starch	Absent
10.	Albumin	Absent
11.	Tannic acid	Absent
12.	Unsaturated compounds	Absent
13.	Reducing sugar	Absent
14.	Amino acid	Absent
15.	Mercury	Absent

From the result of preliminary biochemical analysis reveals that trial drug – *Pavalaveerachunnam* shows the presence of calcium, iron in ferrous form, sulphate, chloride, phosphate, and amino acid.

INTERPRETATION:

CALCIUM:

- Maintenance of plasma calcium level within normal range is of vital importance because neuro-muscular excitability is dependent on plasma calcium level.

- ❖ Normal neuro-muscular excitability maintained by calcium present in the test drug favours aphrodisiac activity

IRON: (Ferrous and Ferric form)

- ❖ Improves general nourishment
- ❖ Improves oxygen carrying capacity of the blood
- ❖ Hence, reduces fatigue and cures anemia
- ❖ Absorbed in ferrous form and converted to ferric form
- ❖ Thus, test drug meets the daily Iron requirement of our body. [male-10mg/day; female-15-20mg/day]

CHLORIDE:

- ❖ Chloride forms the chief anion of the extracellular fluid and exists along with sodium mostly.
- ❖ Regulates acid base balance
- ❖ Formation of HCl in gastric juice
- ❖ Help to preserve normal neuromuscular irritability by maintaining a state of equilibrium, on account of their relative proportion in ECF and ICF.

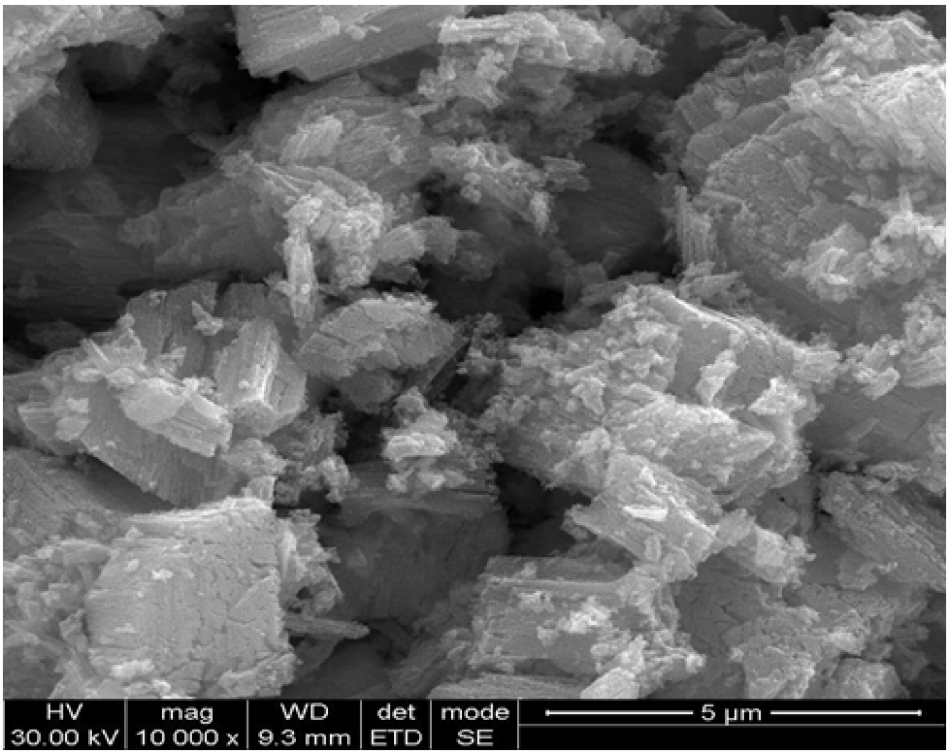
PHOSPHORUS:

- ❖ It involves the function of repair of body cells especially hepatocytes
- ❖ It plays major role in structural frame work of DNA and RNA
- ❖ It is very much important for production of RBC from liver cells
- ❖ Contributes formation of ATP, ADP and creatine phosphate
- ❖ Formation of physiologically important phosphorus containing compounds like phospholipids, co-enzymes, and enzymes of intermediary metabolism.

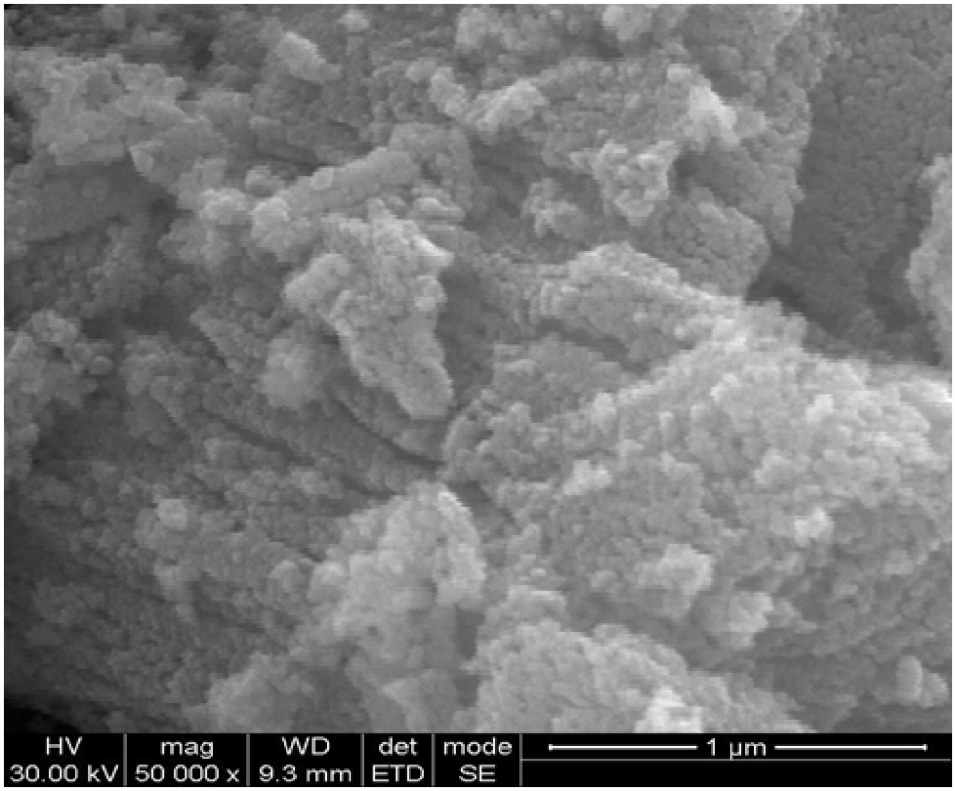
SULPHATE:

- ➢ Nutritionally essential element.
- ➢ Sulphate has anti bacterial activity and it is one of the macronutrient of cells.
- ➢ It inhibits growth of yeasts and moulds in low pH and inhibits growth of enterobacteriae and other gram- negative bacteria in high pH.
- ➢ Sulphate important role for the anti – microbial activity.
- ➢ It is needed to start the cascade of digestive enzymes released from the pancreas. Without proteases, lipases and amylases, food is not digested efficiently.

SEM-Analysis of *Pavalaveerachunnam*



SEM-Analysis of *Pavalaveerachunnam ×10,000 Magnification*



SEM-Analysis of *Pavalaveerachunnam - 50,000 ×Magnification*

Figure No:12 SEM IMAGES OF PVC

Interpretation:

SEM analysis of the ***Pavalaveerachunnam***shows most of the particles present in the sample is nano size, average particle size is 100-150 nano microns. So, very minimal quantity of the medicine is enough to treat the disease.

Siddhars were the great scientist in ancient times. They used nano technology for the preparation of ***Chunnam***. Nano particles have beneficial properties that can be used to improve drug delivery system. Target cells take up these nano particles quickly because of their smaller size, lesser particles enhance the bio absorption and bio availability resulting efficacy of the drug will be increased. Larger particles could not enter in to the target cell because of their size, resulting they cleared from the body. If a drug is cleared too quickly from the body, this could force a patient to use high dose, poor bio distribution is a problem that can affect normal tissue through wide spread distribution but the particles from drug delivery systems lower the volume of distribution and reduce the effect on non-target tissue. Adjuvant and detoxification (Purification) is also important factors for drug transport.

Nano particles are defined as particulate dispersion or solid particles with a size in the range of 1-100nm in diameter.

They are easily

- Absorbable
- Biodegradable
- Biocompatible
- Non-antigenic in nature
- Selective/Targeted/Controlled delivery of drugs to specific site of action in the body even across the blood brain barrier
- Use to extend time window of bioavailability and to protect drug from enzymatic and chemical decomposition
- Result in reduced peripheral side effect of drugs

The nanoparticles present in the drug results in a better bioavailability and facilitates absorption.

ICP – OES (INDUCTIVELY COUPLED PLASMA OPTICAL EMISSION SPECTROSCOPY)

PavalaveeraChunnam(wt:0.31031g)

The drug Pavalaveera chunnam sample was analysed by the ICP-OES to detect the trace elements and there elements quantitatively.

Table No:8 **ICP – OES Result of *Pavalaveera Chunnam***

S.NO	Elements	Wavelength	Concentration
1.	As	188.979	BDL
2.	Ca	315.807	234.150mg/L
3.	Cd	228.802	BDL
4.	Cu	327.393	BDL
5.	Fe	238.204	12.380mg/L
6.	Hg	253.652	3.574mg/L
7.	K	766.491	50.821mg/L
8.	Mg	285.213	02.020mg/L
9.	Na	589.592	02.110mg/L
10.	Ni	231.604	BDL
11.	Pb	220.353	01.254mg/L
12.	P	213.617	58.541mg/L
13.	Si	251.611	01.789mg/L
14.	S	180.731	10.514mg/L
15.	Zn	213.856	01.245mg/L

BDL: Below Detectable Limit

1% =10000 ppm,-

1ppm= 1/1000000 or 1ppm=0.0001%

The toxic metals and the permissible limits

Heavy metals	**WHO&FDA limits**
Arsenic(As)	10ppm
Me rcury (Hg)	1ppm
Lead(Pb)	10ppm
Cadmium(Cd)	0.3ppm

ICP – MS (INDUCTIVELY COUPLED PLASMA OPTICAL MASS SPECTROSCOPY)

PavalaveeraChunnam (wt:0.31031g)

The drug Pavalaveera chunnam sample was analysed by the ICP-MS to detect the trace elements and there elements quantitatively .

Table No:9 **ICP – MS Result of *PavalaveeraChunnam***

Element	Element cone in Blank (pph)	Element cone in Sample (pph)	Actual Elemental cone in sample (sample - blank) (pph)	Actual Elemental cone in sample (ppm)
V	0.0040	4.2870	4.2830	**0.0043**
Hg	0.0040	3.4700	3.4660	**0.0035**
Cr	0.0479	43.1500	43.1021	**0.0431**
Co	0.0122	0.9758	0.9636	**0.0010**
Ni	0.1446	8.0640	7.9194	**0.0079**
Cu	0.1583	13.7330	13.5747	**0.0136**
As	0.0326	20.2190	20.1864	**0.0202**
Mo	0.0108	0.7833	0.7725	**0.0008**
Ru	0.0040	0.0028	-0.0012	**0.0000**
Rh	0.0010	0.0256	0.0246	**0.0000**
Pd	0.0071	0.6990	0.6918	**0.0007**
Cd	0.0110	0.0312	0.0202	**0.0000**
Os	0.0032	0.0032	0.0000	**0.0000**
Ir	0.0176	0.0000	-0.0176	**0.0000**
Pt	0.0445	0.0000	-0.0445	**0.0000**
Au	0.3437	25.8080	25.4643	**0.0255**
Pb	0.1358	1.4800	1.3442	**0.0013**

BDL: Below Detectable Limit

1% =10000 ppm,-

1ppm= 1/1000000 or 1ppm=0.0001%

The toxic metals and the permissible limits

Heavy metals	**WHO&FDA limits**
Arsenic(As)	10ppm
Me rcury (Hg)	1ppm
Lead(Pb)	10ppm
Cadmium(Cd)	0.3ppm

Interpretation:

The result indicate that the formulation is extremely safe as it contains heavy metals within specified limits.

ICP-OES,ICP-MS reveals high concentration of Sodium in *pavalaveera chunnam* (589.592 mg/L). It also has physiologically important minerals like Calcium, Phosphorus and Potassium. In *pavalaveera chunnam*, the heavy metals like Arsenic, Mercury, Lead, Cadmium and trace element like Nikkal were below detectable level. This reveals the safety of the drug and it has free from toxic substances and has no side effects. .

Sodium:

- Sodium regulates the acid-base balance of the body fluids.
- Sodium is required for the maintenance of osmotic pressure of the body fluids.
- Sodium is involved in the intestinal absorption of glucose and amino acids.
- It is necessary for the normal muscle irritability and permeability of cells.
- Sodium maintains extracellular osmotic pressure.

Potassium:

- Potassium is required for the regulation of acid-base balance and water balance of the body fluids.
- Potassium maintains intracellular osmotic pressure.

Calcium:

- Calcium ions are necessary for the maintenance and regulation of acid-base balance and water balance in the body.
- Calcium influences the membrane structure and transport of water and several ions across it.

Phosphorus:

- Phosphorus plays an important role for the formation and utilization of high-energy production

- Phosphorus involves the function of repair of body cells specially hepto cells and tissues.
- Phosphate buffer system is important for the maintenance of pH in the blood(7.4)as well as the cells.

FTIR: (FOURIER TRANSFORM INFRA-RED SPECTROSCOPY)

Fourier Transform Infra-Red Spectroscopy (FTIR) analysis results in absorption spectra provide information about the functional group and molecular structure of a material. In FTIR the wavenumbers between 4000cml - 600 cral is known as functional group area. <600cml wavenumbers is known as fingerprint area. The corresponding absorption frequency by FTIR shows the presence of alcohol, phenol, Beneze, 1°, 2°amines, amides, alkanes, 1° amines, aromatics, aliphatic amines and alkyl halides.

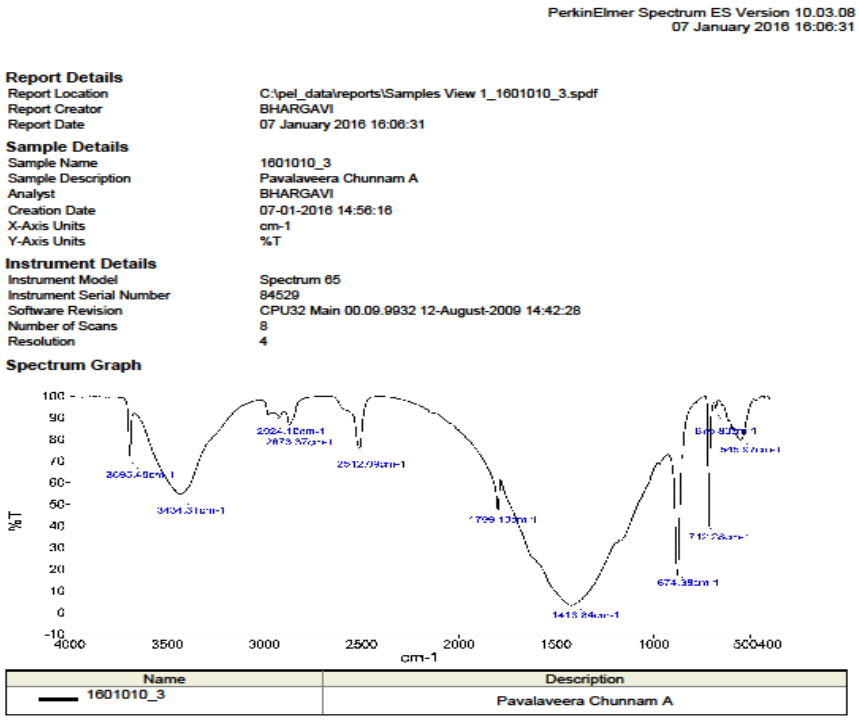


Figure No:13 FTIR IMAGE OF *PAVALAVEERA CHUNNAM*

FTIR is a very useful tool in the recognition of the functional groups of bio molecules, thus aiding in their structural elucidation, so confirming the presence of active molecules responsible for the therapeutic activity of Siddha drugs.

FUNCTIONAL GROUPS PRESETS IN *Pavalaveerachunnam*

Table no: 11 FTIR Spectrum-functional groups

Frequency (cm)	Bond	Functional groups
545.95	C – X	Chloro alkanes
675.80	C – H	Vinyl - Trans - disubstituted alkanes
712.28	C – H	Aromatic - mono substituted benzene,
		potassium persulfate,copper
1418.84	S = O	sulfate
1799.10	C - O	carboxylic acid - acyl halides
2512.09	N - H	Ammonium ions
2873.37	N - H	Ammonium ions
2924.10	N - H	Ammonium ions
3434.31 -	N - H	Primary amines
3695.49	O - H.	Alcohols,Phenols

Benzene:

- Benzene acts as a diuretic
- Benzene is natural consitutent of crude oil. Benzene is colourless,flammable liquid with sweet odor.
- It is used as a starting material in making other chemicals,including plastics,lubricants rubbers,dyes,detergents,drugs and pesticides.
- Toluence is often used as a substance for benzene.Toluence is also processed into benzene.

Salts:

- Salts are very soluble in water.
- Sulfates are salts of sulfuric acid.
- Sulfate reducing bacteria,some anaerobic microorganisms.

Alkyline Group:

- In a unusual process,ammonium ions from an amalgam.Such species are prepared by the electrolysis of an ammonium solution using a mercury cathode.

- ❖ Nice powder,white colour,Tasteless and no-significant smell indicates the complete calcination process.
- ❖ Soluable in acids shows easily absoption in stomach.

Phenolic groups

- ❖ It acts as neuro transmitters
- ❖ This group of substance has antimicrobial,antiseptic and antioxidant activities

Alkanes groups

- ❖ Alkane have little biological activity
- ❖ It is predominate in plants.They protect against bacteria and fungi.

ACUTE TOXICITY STUDY

Acute toxicity studies were performed according to organization for economic cooperation and development (OECD) guidelines 423 (Ecobichon and Zelt, 1979). Male rats were selected by random sampling technique employed in this study. The animals were fasted for 4 hours with free access to water only. The different doses of the extract were administered orally morbitity and mortality if any was observed for 3 days. If mortality was observed in two out of three animals, then the dose administrated was considered as toxic dose. If the mortality was observed in only one animal out of three animals then the same dose was repeated again to confirm the toxic effect. If no mortality was observed then selected doses of the extract were employed for further toxicity studies.

In the present study, even 2000 mg/kg dose of ***Pavalaveera chunnam*** did not produce any mortality and morbidity. The results of different parameters observed during acute toxicity study are tabulated in table.12

Therefore the biological evaluation was carried out at a very conventionally safer range of 200 and 400mg/kg doses

Table No.:12 Effect of *Pavalaveera chunnam* on Acute toxicity

Dose mg/kg	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
2000	−	−	−	−	+	+	−	−	−	−	−	−	+	−	−	−	−	−	−	+	+	+	+	−

indicates presence, -: indicates absence Sub chronic Toxicity Studies

Study Parameters Observation

1.Skin, 2.Fur, 3.Eyes, 4.Grooming, 5.Apathy, 6.Ataxia, 7.Circling, 8.Compulsive, 9.Locomotor Activity, 10.Alertness, 11.Depression, 12.Paralisis, 13.Touch response, 14.Diarrhoea, 15.Salivation, 16.Lacrimation, 17.Piloerection, 18.Convulsion, 19.Tremor, 20.Grip strength, 21.Righting reflex, 22.Corrneal Reflex, 23. Sleep, 24.Coma

In the present study showed even 2000mg/kg dose of VP did not produce any mortality and morbidity. The results of different parameters observed during acute toxicity study are tabulated in table No:12

Therefore the biological evaluation was carried out at a very conventionally safer range of l00mg, 200mg, 400mg/kg doses

The acute oral toxicity potentials of ***Pavalaveera Chunnnam*** in female Wistar albino rats were studied effectively.

The acute toxicity result shows the test drug VP does not produce any toxic signs and mortality up to its maximum dose level of 2000 mg/animal body weight in wister albino rats.

Morphological characters like changes in skin, eyes, fur and nose appeared normal.

It showed changes in touch response and decreased motor activity. The behavioural changes are normal.

According to OECD guidelines, for acute oral toxicity LDso dose upto 2000mg/ kg of the drug the test drug ***Pavalaveera Chunnnam*** is a safe herbo mineral drug and can be used for long time administration.

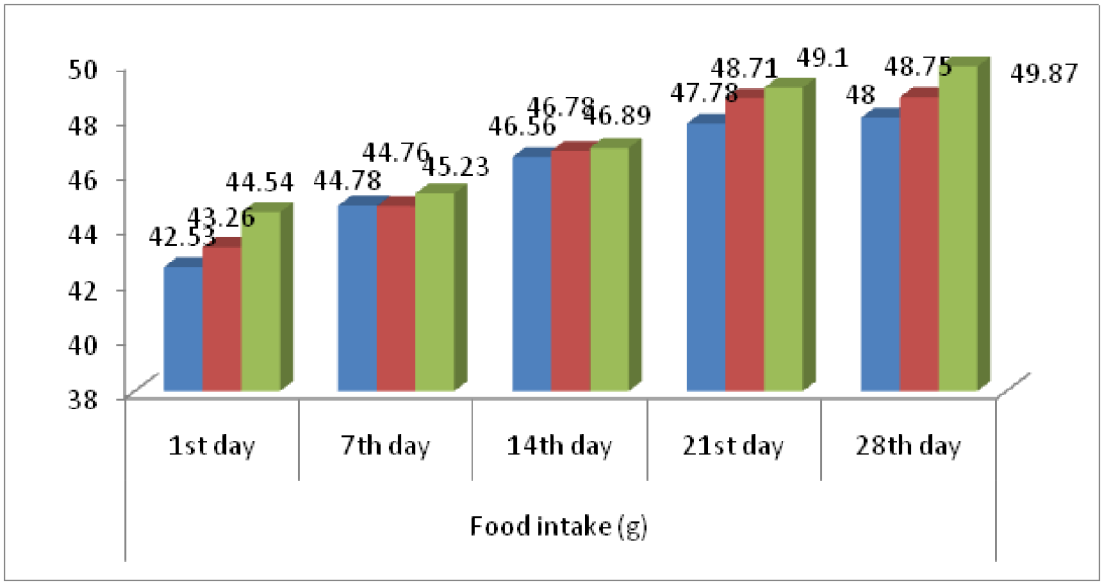
SUB ACUTE TOXICITY STUDY

Average Feed intake during sub-acute toxicity study of *Pavalaveera chunnam*

The average feed consumption was analyzed in group II and III animals in days (1,7, 14,21 and 28) and compared with the group I control animals. The results are presented in Table 11. and in the accompanying figure. The average food consumption increased significantly in all the groups from day 1 to 29 day. The values are expressed in figure

TableNo:13 Average Feed intake during sub-acute toxicity study of PVC

Group No.	Drug Treatment	Dose (mg/kg) p.o	Food intake (g)				
			1st day	7th day	14th day	21st day	28th day
1.	Control (Normal saline)	10ml/kg	42.53±1.30	44.78±2.10	46.56±2.30	47.78±2.38	48±1.18
2.	Drug	200	43.26±1.29	44.76±2.31	46.78±2.10	48.71±2.34	48.75±1.34
3.	Drug	400	44.54±1.38	45.23±1.45	46.89±1.98	49.10±1.32	49.87±1.68



Group I Control

Group II Low dose of PVC (200mg /kg)

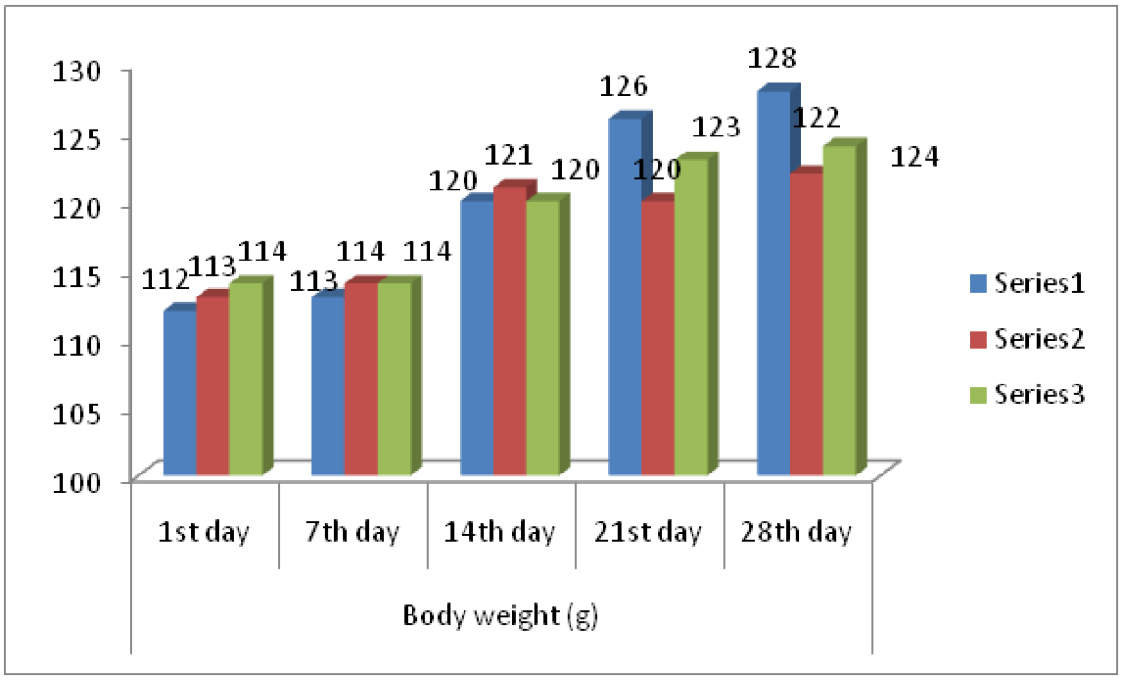
Group III Medium dose of PVC (400mg/kg)

Effect of Body Weight during sub-Chronic toxicity study of *Pavalaveera Chunnam*

The body weight of the different treatment groups were also estimated on the days (1,7,14,21 and 28) to assess the toxicity of Pavalaveera chunnam. The different treatment group animals shows significant increase in body weight starting from day 1 to day 29 was observed, (figure)

Table No 13:Average Body weight during sub-acute toxicity study of PVC

Group No.	Drug Treatment	Dose (mg/kg)p.o	Body weight (g)				
			1st day	7th day	14th day	21st day	28th day
1.	Control (Normal saline)	10ml/kg	112±1.60	113± 1.50	120±1.3	126±1.7	128±1.6
2.	Drug	200	113±1.70	114±1.20	121±1.70	120±1.90	122±1.50
3.	Drug	400	114±1.60	114±1.60	120±1.90	123±1.70	124±1.70



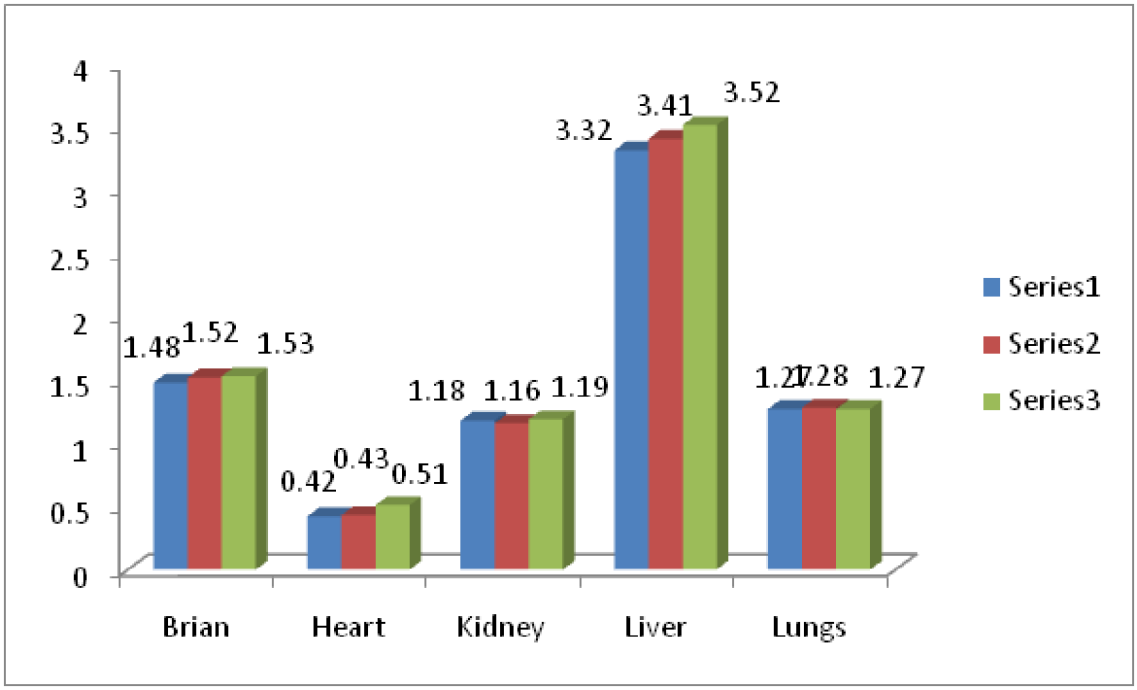
Group I Control
Group II Low dose of PVC (200mg /kg)
Group III Medium dose of PVC (400mg/kg)

Effect of organ weight during sub-acute toxicity study of *Pavalaveera chunnam*

The rate of size of organ per 100 grams of body weight are presented in the table Administration of ***Pavalaveera chunnam*** different doses did not alter significantly the organ weights in group II and III. A mild increase in the weight of lung and kidneys was observed in Group III animals which were treated with 400mg / kg of ***Pavalaveera chunnam***

Table No14:Average organ weight during sub-acute toxicity study of PVC

Group No	Drug Treatment	Dose (mg/kg p.o)	Brian	Heart	Kidney	Liver	Lungs
1.	Control (Nornal saline)	10ml/kg	1.48 ± 0.21	0.42=0.03	1.18 ± 0.08	3.32 ± 0.42	1.27 ± 0.08
2	Drug	200	1.52 ± 0.07	0.43=0.05	1.16 ±0.04	3.41 ±0.53	1.28 ±0.07
3	Drug	400	1.53 ± 0.09	0.51=0.06	1.19± 0.07	3.52 ±0.36	1.27± 0.10



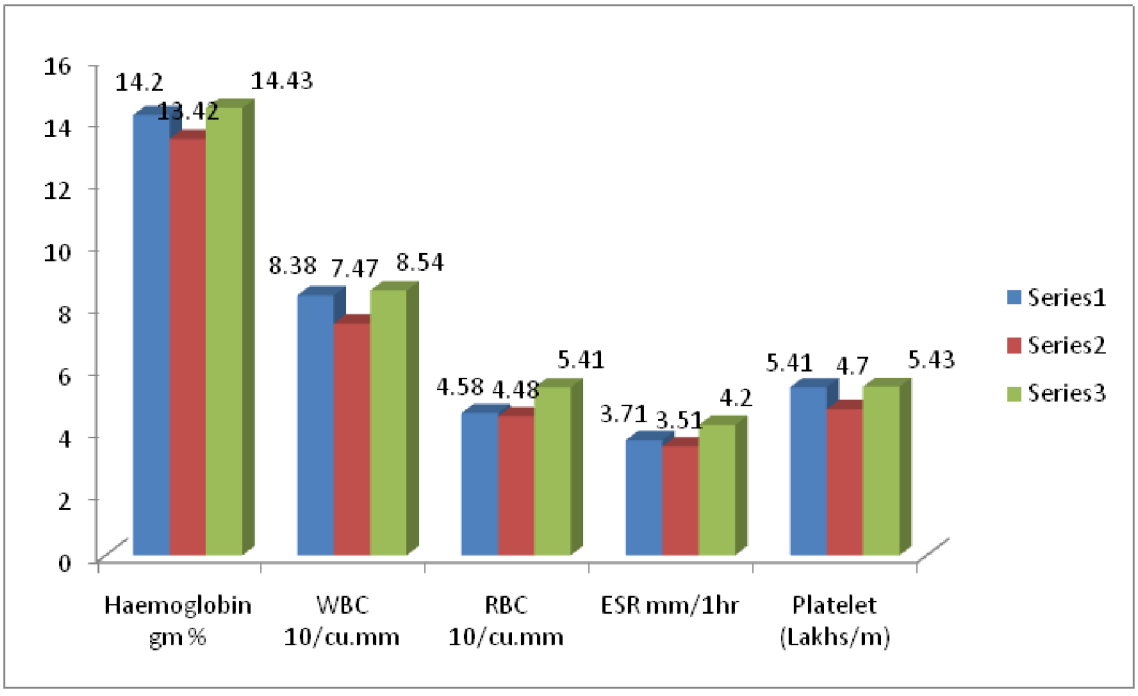
Group I Control
Group II Low dose of PVC (200mg /kg)
Group III Medium dose of PVC (400mg/kg)

Effect of *Pavalaveera chunnam* on Hematological parameters during sub-acute toxicity

The hematological parameters (RBC, WBC, Platelets and Hb) were estimated in the different groups treated with Pavalaveera chunnam and were compared with the control animals (Group I). The WBCs increased significantly in Group III animals when compared with Group I. The Hb showed a significant increase in group III animals. Interestingly RBC count was significantly increased in Group III animals. The values are tabulated in ***Table15***

Table No 15:Average Hematological parameters during sub-acute toxicity study of PVC

Group No.	Drug Treatment	Dose (mg/kg p.o)	Haemoglobin gm %	WBC 10/cu.mm	RBC 10/cu.mm	ESR mm/1hr	Platelet (Lakhs/m)
1.	Control (Normal saline)	10ml/kg	14.2 ± 0.60	8.38 ± 0.50	4.58 ± 0.70	3.71± 0.50	5.41 ± 0.80
2.	Drug	200	13.42±0.50	7.47±0.80	4.48±0.90	3.51± 0.03	4.70± 0.70
3.	Drug	400	14.43± 0.30	8.54±0.70	5.41± 0.80	4.20± 0.20	5.43± 0.50



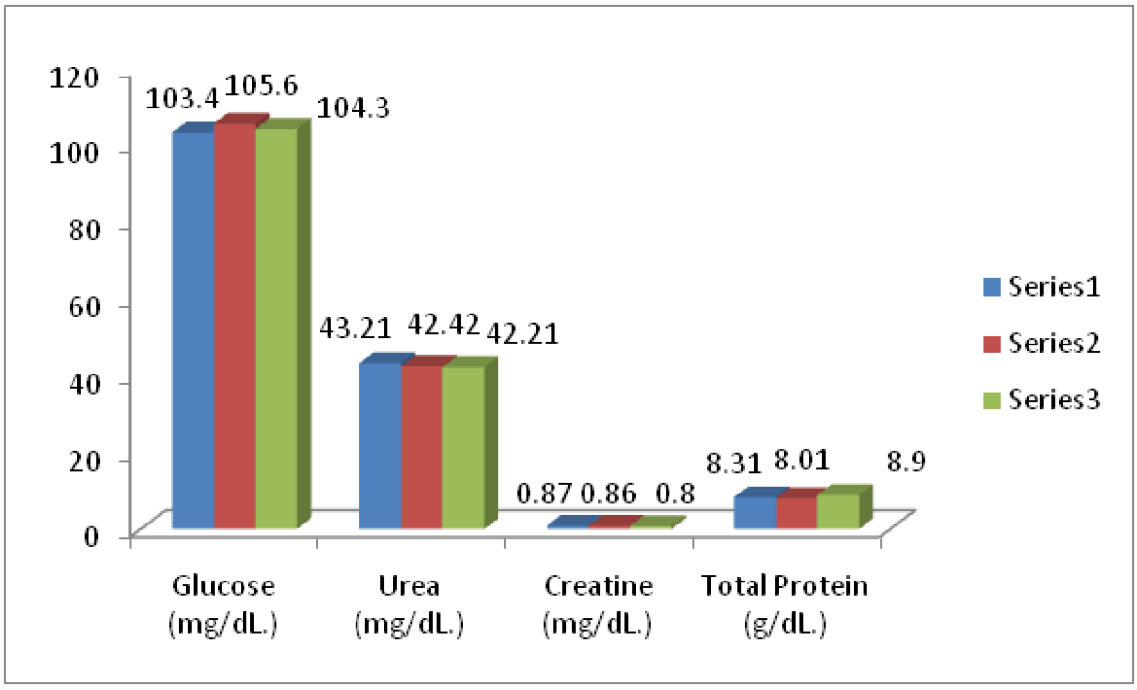
Group I Control
Group II Low dose of PVC (200mg /kg)
Group III Medium dose of PVC (400mg/kg)

Effect of *Pavalaveera chunnam* on Renal parameters in experimental rats.

The effect of ***Pavalaveera chunnam*** administration in kidney function was also analyzed by estimating the levels of Glucose, Urea, creatinine and Protein the results are presented in table Administration of ***Pavala veera chunnam*** did not alter the kidney function significantly. The values are shown in Table 16.

Table No 16:Average Renal parameters during sub-acute toxicity study of PVC

Group No.	Drug Treatment	Dose (mg/kg) p.o	Glucose (mg/dL.)	Urea (mg/dL.)	Creatine (mg/dL.)	Total Protein (g/dL.)
1.	Control (Normal Saline)	10ml/kg	103.4±1.34	43.21±1.23	0.87± 0.78	8.31± 0.65
2.	Drug	200	105.6±1.65	42.42±1.61	0.86±0.84	8.01± 0.31
3.	Drug	400	104.3±1.43	42.21±1.34	0.80± 0.84	8.90± 0.67



Group I Control

Group II Low dose of PVC (200mg /kg)

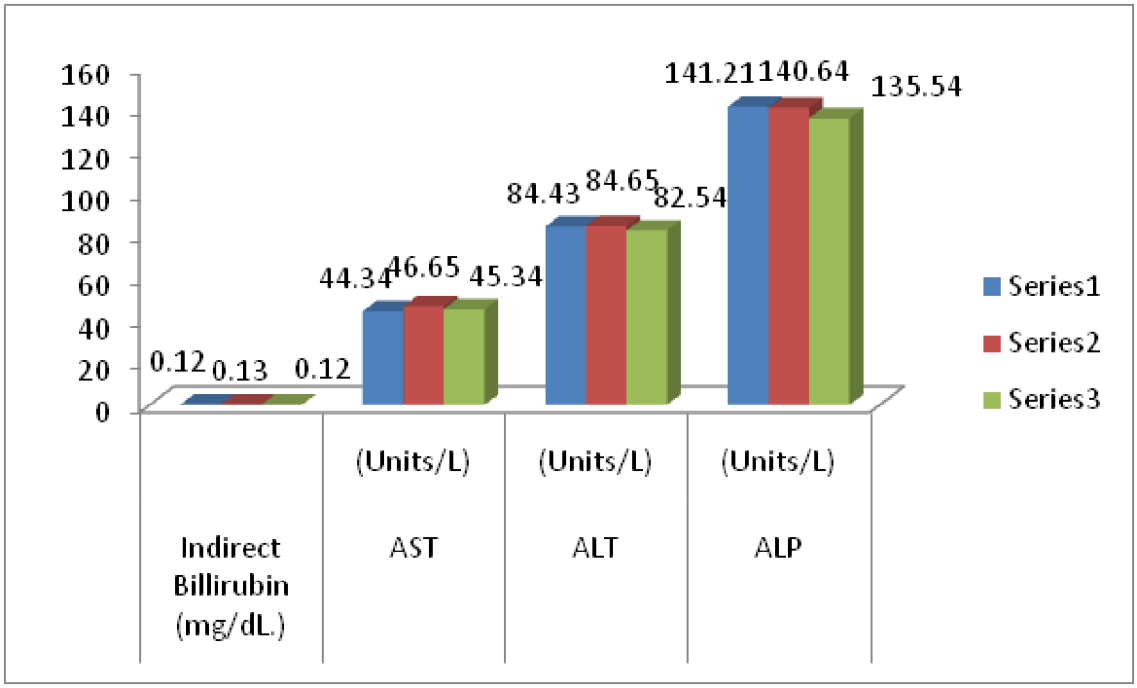
Group III Medium dose of PVC (400mg/kg)

Effect of *Pavalaveera chunnam* on Liver parameters in experimental rats.

The effect of ***Pavalaveera chunnam*** in the liver function was analyzed by estimating the levels of key enzymes namely AST, ALT, ALP and also the Bilirubin. The results are presented in table. The administration of ***Pavalaveera chunnam*** did alter the liver function.The total bilirubin levels didn't show any significant increase in all the groups when compared to the group I animals.

Table No : 17 Average Liver parameters during sub-acute toxicity study of PVC

Group No.	Drug Treatment	Dose (mg/kg p.o)	Indirect Billirubin (mg/dL.)	AST (Units/L)	ALT (Units/L)	ALP (Units/L)
1.	Control (Normal Saline)	100ml/Kg	0.12±0.01	44.34± 1.31	84.43± 2.32	141.21± 1.23
2	.Drug	200	0.13± 0.02	46.65± 2.34	84.65± 2.12	140.64 ± 1.72
3.	Drug	400	0.12± 0.02	45.34±2.14	82.54± 2.43	135.54± 1.45



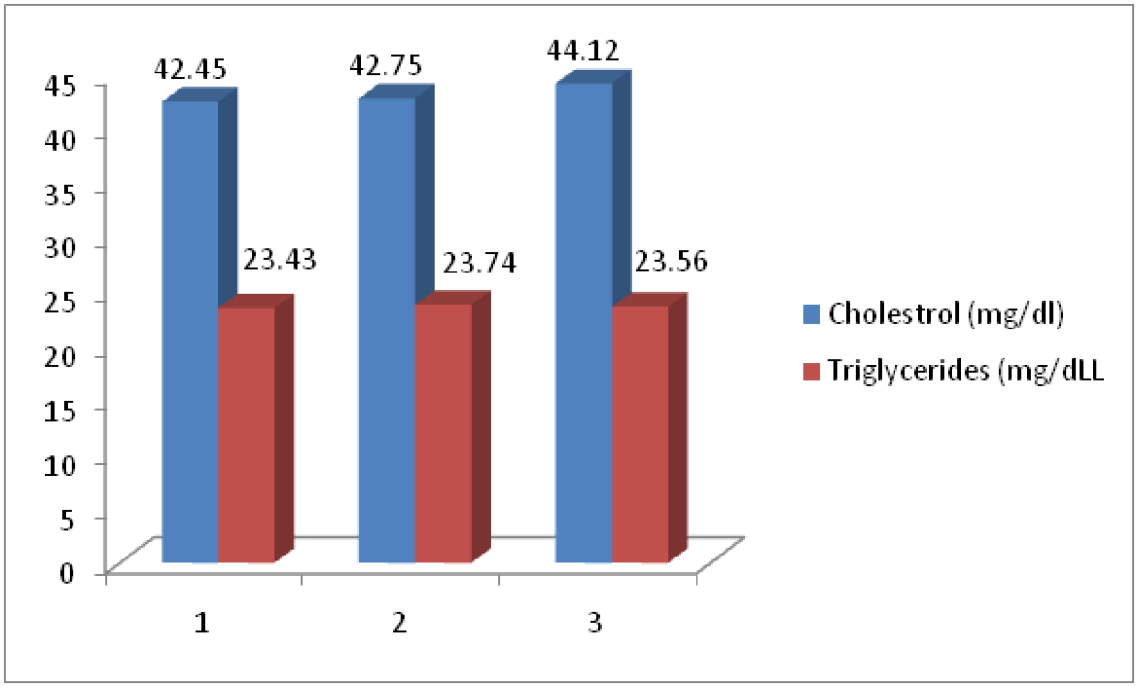
Group I Control
Group II Low dose of PVC (200mg /kg)
Group III Medium dose of PVC (400mg/kg)

Effects of *Pavalaveerachurnam* on biochemical parameters of sub-acute toxicity studies in albino rats.

The effect of ***Pavalaveera chunnam*** *in the* biochemical parameters was analyzed by estimating the levels of key enzymes namely Cholesterol and Triglycerides . The results are presented in table. The administration of ***Pavalaveera chunnam*** did alter the Cholesterol level.

Table No:18 Average biochemical parameters during sub-acute toxicity study of PVC

Group No.	Drug Treatment	Dose (mg/kg)	Cholestrol (mg/dl)	Triglycerides (mg/dLL
1.	Control(saline)	10 ml/kg	42.45+ 2.32	23.43+ 2.14
2.	Drug	200	42.75+ 1.78	23.74+ 1.97
3.	Drug	400	44.12+ 1.34	23.56+ 1.4



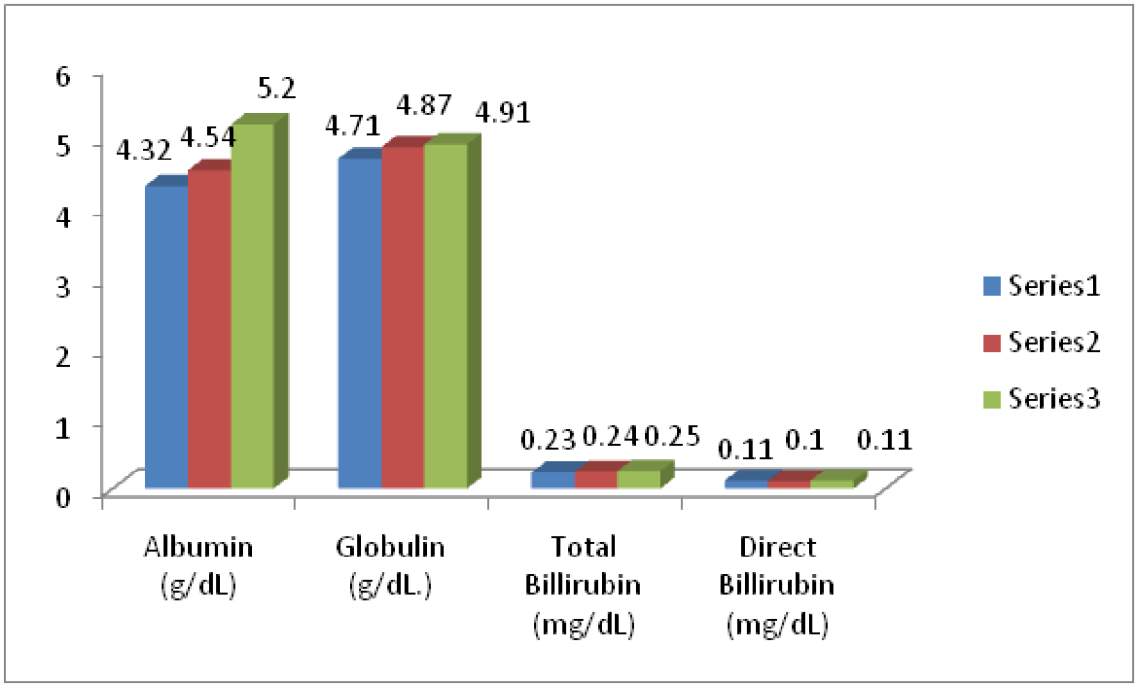
Group I Control
Group II Low dose of PVC (200mg /kg)
Group III Medium dose of PVC (400mg/kg)

Effects of *Pavalaveerachurnam* on biochemical parameters of sub acute toxicity studies in albino rats.

The effect of ***Pavalaveera chunnam*** in the biochemical parameters was analyzed by estimating the levels of key enzymes namely Albumin, Globulin, Total Bilirubin and Direct Bilirubin . The results are presented in table. The administration of ***Pavalaveera chunnam*** did alter the Albumin, Globulin, Total Bilirubin and Direct Bilirubin level.

Table No:19 Effect of *Pavalaveera chunnam* on biochemical parameter of sub-acute toxicity studies in albino rats.

Group No.	Drug Treatment	Dose (mg/kg p.o)	Albumin (g/dL)	Globulin (g/dL.)	Total Billirubin (mg/dL)	Direct Billirubin (mg/dL)
1.	Control (Normal Saline)	10ml/kg	4.32± 1.06	4.71± 1.05	0.23± 0.01	0.11± 0.01
2.	Drug	200	4.54± 0.20	4.87± 0.87	0.24± 0.02	0.10± 0.01
3.	Drug	400	5.20± 0.78	4.91±0.92	0.25± 0.01	0.11± 0.01



Group I Control
Group II Low dose of PVC (200mg /kg)
Group III Medium dose of PVC (400mg/kg)

Table No 15: Effect of PVC on Histopathological study

DOSES	*ORGANS*	*MICRO FATTY*	*FIBROSIS*	*CONGESTION*
LOW DOSE	**SPLEEN**	**NRC**	**NIL**	**NIL**
	HEART	**NRC**	**NIL**	**NIL**
	LIVER	**NRC**	**NIL**	**NIL**
	KIDNEY	**NRC**	**NIL**	**NIL**
HIGH DOSE	**SPLEEN**	**NRC**	**NIL**	**NIL**
	HEART	**NRC**	**NIL**	**NIL**
	LIVER	**NRC**	**NIL**	**NIL**
	KIDNEY	**NRC**	**NIL**	**NIL**

Interpretation:

Histopathology analysis of heart, liver, kidney shown in figure No 15. Gross pathological examination of animals does not reveal any abnormalities in control and test groups. Heart, Liver, Spleen and Kidney showed normal histopathology.

All other organs tested in this study showed normal architecture when compared with control.

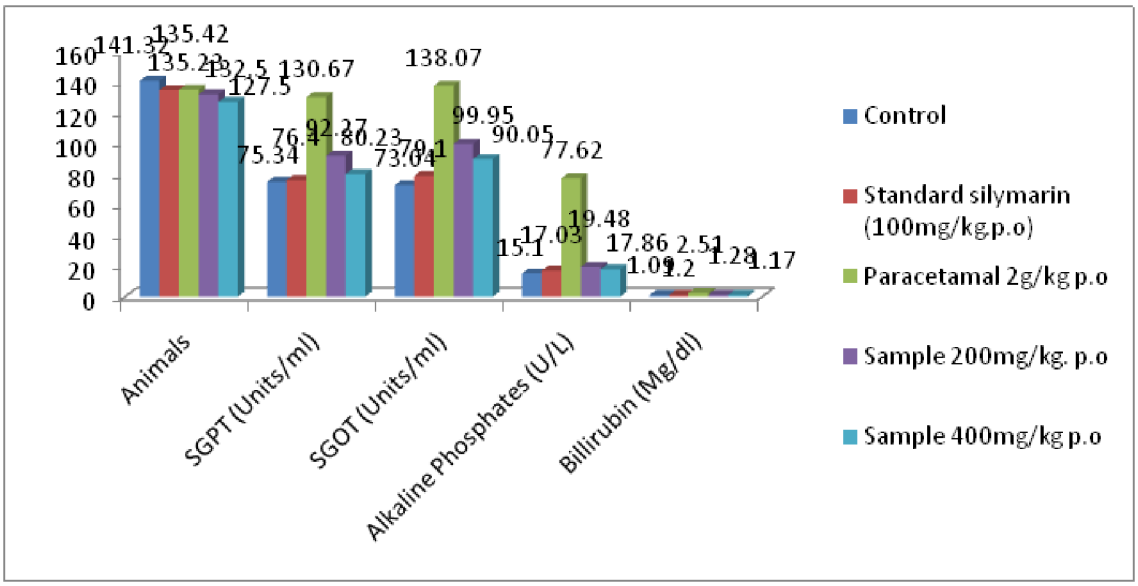
PHARMACOLOGIGAL STUDY

HEPATOPROTECTIVE ACTIVITY OF *PAVALAVEERA CHUNNAM*

Hepatoprotective Activity of *PavalaVeera Chunnam* Paracetamol- induced Liver damage in Rats

Table No. 20 Hepatoprotective Activity of Pavalaveera Chunnam

	Animals	SGPT (Units/ml)	SGOT (Units/ml)	Alkaline Phosphates (U/L)	Billirubin (Mg/dl)
Control	141.32±3.25	75.34±2.15	73.04±1.35	15.1±0.65	1.09±0.35
Standard silymarin (100mg/kg.p.o)	135.23±2.12	76.40±1.50	79.10±2.31	17.03±0.21	1.20±0.22
Paracetamal 2g/kg p.o	135.42±2.13	130.67±2.31	138.07±3.21	77.62±2.12	2.51±0.31
Sample 200mg/kg. p.o	132.50±3.22	92.27±3.12	99.95±1.31	19.48±1.23	1.28±0.12
Sample 400mg/kg p.o	127.50±5.31	80.23±3.51	90.05±3.21	17.86±1.61	1.17±0.51



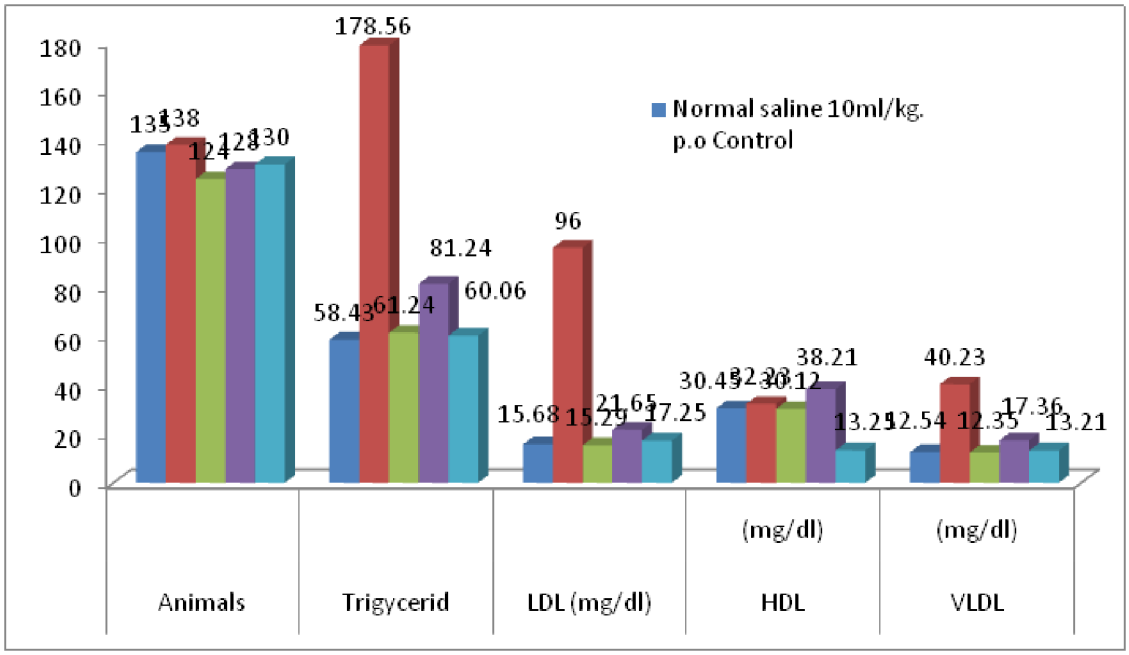
Interpretation:

Liver function test was increased significantly when compared with control group. The animal which is treated with 400mg of test drug PVC the level of LFT was increased significantly. It is concluded that the test drug has significant heptoprotective activity.

HYPOLIPIDEMIC ACTIVITY

Table No. 23 Hypolipidemic activity of Pavalaveera chunnam

S.No	Group	Animals	Trigycerid	LDL (mg/dl)	HDL (mg/dl)	VLDL (mg/dl)
1.	Normal saline 10ml/kg. p.o Control	135±4.54	58.43±1.23	15.68±1.23	30.45± 1.23	12.54± 1.17
2.	Positive control 400 mg/kg(cholestoral induced	138±5.21	178.56± 2.31	96± 2.21	32.23± 2.32	40.23± 2.31
3	Group II + Atorvastain 13mg/kg p.o	124±3.52	61.24± 1.30	15.29±2.31	30.12± 1.09	12.35± 1.19
4.	Group II + sample 200mg/kg p.o	128± 2.10	81.24± 1.20	21.65± 2.34	38.21± 3.43	17.36± 1.26
5.	Group II + Sample 400mg / kg p.o	130±1.27	60.06± 2.12	17.25± 1.24	13.25± 2.28	13.21± 1.42



The mice fed with high cholesterol diet for 7 days exhibited significant increase in serum TG, LDL and VLDL and decrease in HDL as compared to normal diet control group. Treatment with ***Pavalaveera Chunnam*** (200mg,400mg/kg body weight) showed significant decrease in elevated TG, LDL, and VLDL, with significant increase in HDL as

compared to high cholesterol diet control. This lipid lowering effect may be due to the inhibition of hepatic cholesterogenesis or due to the increase in excretion of fecal sterol (Purohit and Vyas.,2006). Plant sterols are also reported to decrease cholesterol absorption and they compete with dietary and biliary cholesterol for incorporation into mixed miscelles in the intestinal lumen thus inhibiting their uptake (Brufau et al., 2008). Hyperlipidemia is associated with the heart diseases, which is the leading cause of death in the world.

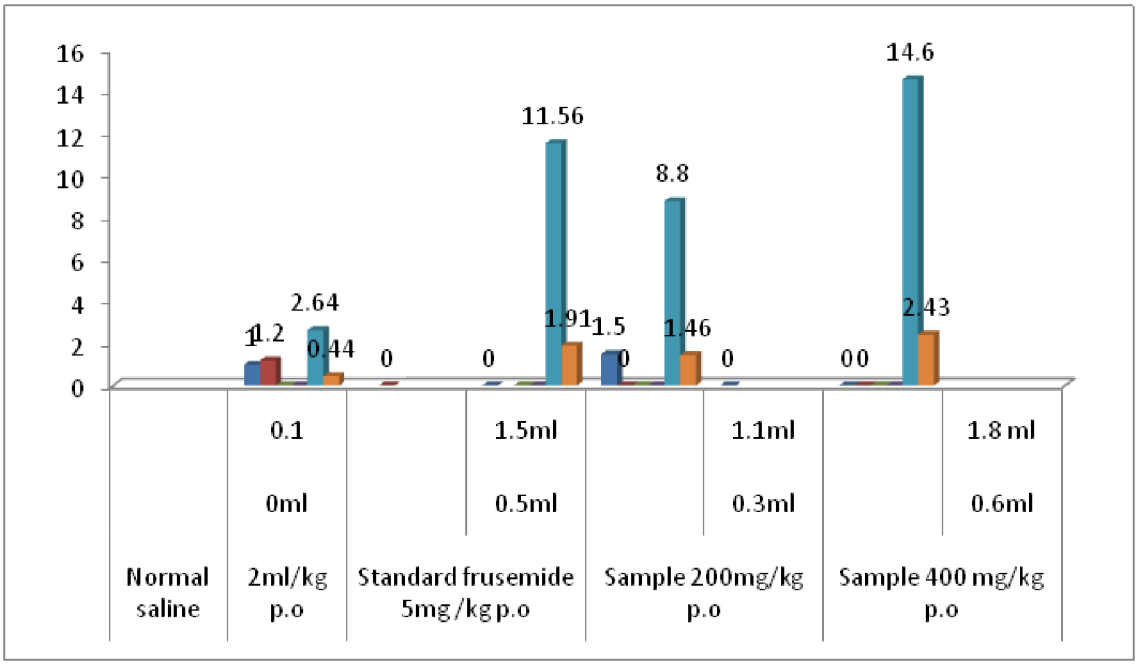
Interpretation:

The investigation of lipid lowering activity on Traditional medicines will be useful strategy in the discovery of new molecules eliciting improved activity by regulating through different mechanism of action.). Thus it is concluded that the test drug *Pavalaveera Chunnam* has significant Hypolipidemic activity.

DIURETIC ACTIVITY (Lipschitz test)]

Table No. 21 Diuretic Activity of Pavalaveera chunnam

Group No	Animal	Drugs	Amount of Urine Collected						Total Volume (ml)	Means
			15mts (ml)	30mts (ml)	1hr (ml)	2hr (ml)	3hr (ml)	4hr (ml)		
I	124.30± 2.40	Normal saline 2ml/kg p.o	0ml	0.10	1.0	1.2	0.14ml	0.2ml	2.64	0.44
II	125.25± 3.32	Standard frusemide 5mg /kg p.o	0.5ml	1.5ml	2ml	2.2ml	2.6 ml	2.7ml	11.56	1.91
III	130.35± 5.20	Sample 200mg/kg p.o	0.3ml	1.1ml	1.5 ml	1.8ml	2 ml	2.1 ml	8.8	1.46
IV	133.26± 3.54	Sample 400 mg/kg p.o	0.6ml	1.8 ml	2.5 ml	3.1 ml	3.4 ml	3.2 ml	14.6	2.43



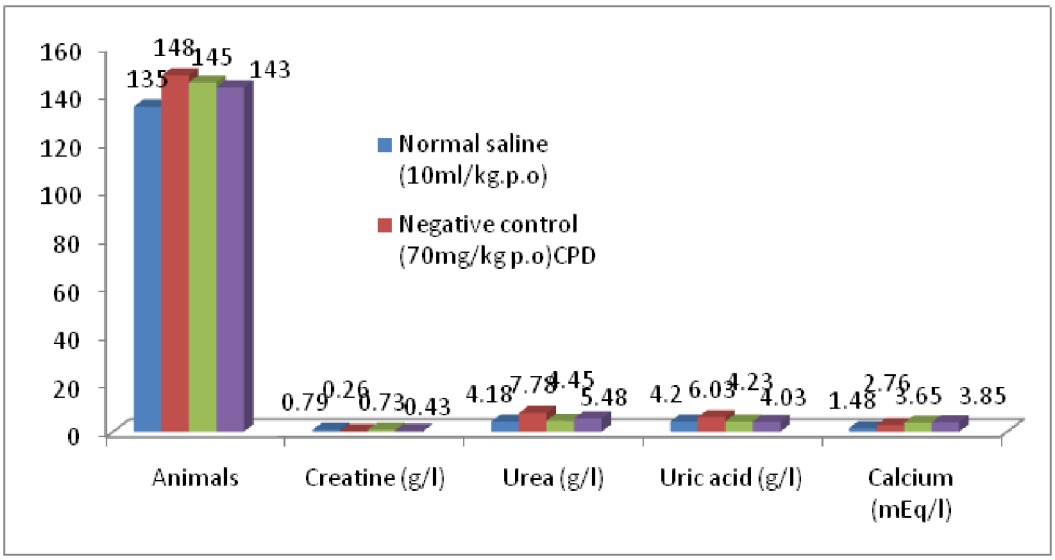
Interpretation:

Urinary excretion of the experimental animals was increased significantly when compared with control group (2.64 ml). In group IV animals the urinary excretion shows significant increase about 14.6ml. It is concluded that the test drug has significant Diuretic activity.

LITHOTRIPTIC ACTIVITY OF *PAVALAVEERA CHUNNAM*

Table No. 22 Lithotriptic activity of *Pavalaveera chunnam*

Group	Urine levels of				
	Animals	Creatine (g/l)	Urea (g/l)	Uric acid (g/l)	Calcium (mEq/l)
Normal saline (10ml/kg.p.o)	135± 2.35	0.79± 0.01	4.18± 0.56	4.2± 0.97	1.48± 0.45
Negative control (70mg/kg p.o)CPD	148±3.12	0.26±0.02	7.78±0.98	6.03±0.12	2.76± 0.93
Cystone (500mg/kg. p.o)	145±2.56	0.73±0.03	4.45±0.02	4.23±0.12	3.65± 0.08
Sample (200mg/kg.p.o)	143± 2.56	0.43± 0.01	5.48±0.02	4.03±0.01	3.85±0.43
Sample (400mg/kg.p.o)					



Group I Normal saline Control

Group II Positive control (400mg/kg.bw) Cholestrol induced diet,

Group III Group II + Atorvastatin (13mg/kg.p.o)

Group IV Group II + Sample drug 200mg/kg.P.O.,

Group V Group II + Sample drug 400mg / kg.P.O.

Interpretation:

Uurinary excretion of stone forming constituents in the experimental animals. The calcium level was increased significantly when compared with control group (1.48mEg). In group IV animals which is treated with 400mg of these drug (Pavalaveera chunnam) the calcium level was increased significantly (4.85mEg). It is concluded that the dest drug has significant **Lithotriptic** activity.

ANTIMICROBIAL ACTIVITY

Table 18: Results of Antimicrobial activity of ***Pavalaveera chunnam***

Micro organism	Zone of inhibition in mm		Inference
	Standard drug (Amaikacin) 50 (ig/disc	Test drug (VP) 50fig/disc	
Klebsiella pneumonia	16	-	Resistant
Salmonella Typhi	17	-	Resistant
P. aeruginosa	16	-	Resistant
E. coli	16	-	Resistant
Staphylococcus albus	16	10	Sensitive
Salmonella typhi	17	-	Resistant
Proteus vulgaris	15	-	Resistant
Streptococcus pyogenes	15	10	Sensitive

Micro organism	Zone of inhibition in mm		Inference
	Standard drug (Ketokonazole) 50 ug/disc	Test drug (VP) 50u.g/disc	
Candida albicans	16	-	Resistant

Interpretation:

From the above results, the test drug (PVC-50mcg) is resistant against ***Klebsiella penumoniae, P.aeruginosa, E.coli, Salmonella typhi*** and ***Candida albicans*** sensitive against ***Staphylococcus aureus,*** and ***Streptococcus pyogenus.*** Sensitive when compared to the standard drug (Amikacin-50mcg) and (Ketokonazole) which was evident from the zone of inhibition.

As the above bacterial organisms responsible for pneumonia, bronchitis, pharyngitis, diarrhoea, typhoid fever, headache, urinary tract infection and skin infectons.

And fungal organism ***Candida albicans*** responsible for superficial skin and mucosal infection. Our results confirmed the traditional use of PVC has antimicrobial activity.

Microbiological Results of *Pavalaveerachunnam*

Staphylococcus albusor Staphylococcusepidermidis is a Gram-positive bacterium, and one of over 40 species belonging to the genus Staphylococcus.It is part of the normal human flora, typically the skin flora, and less commonly the mucosal flora. Although *S. epidermidis* is not usually pathogenic, patients with compromised immune systems are at risk of developing infection. These infections are generally hospital-acquired. *S. epidermidis* is a particular concern for people with catheters or other surgical implants because it is known to formbiofilms that grow on these devices. Being part of the normal skin flora, *S. epidermidis* is a frequent contaminant of specimens sent to the diagnostic laboratory. ***S. epidermidis*** causes biofilms to grow on plastic devices placed within the body. This occurs most commonly on intravenous catheters and on medical prostheses. Infection can also occur in dialysis patients or anyone with an implanted plastic device that may have been contaminated. It also causes endocarditis, most often in patients with defective heart valves. In some other cases, sepsis can occur in hospital patients.

Streptococcus pyogenes is a species of bacteria. Like most other streptococci, it is clinically important in human illness. It is an infrequent, but usually pathogenic, part of the skin flora. It is the sole species of Lancefield group A and is often called **group A streptococcus** (**GAS**),

S. pyogenes is the cause of many important human diseases, ranging from mild superficial skin infections to life-threatening systemic diseases.Infections typically begin in the throat or skin. The most striking sign is a strawberry-like rash. Examples of mild *S.pyogenes* infections include pharyngitis (strep throat) and localized skin infection (impetigo).Erysipelas and cellulitis are characterized by multiplication and lateral spread of *S. pyogenes* in deep layers of the skin. *S. pyogenes* invasion and multiplication in the fascia can lead to necrotizing fasciitis, a life-threatening condition requiring surgery.Infections due to certain strains of *S. pyogenes* can be associated with the release of bacterial toxins. Throat infections associated with release of certain toxins lead to scarlet fever. Other toxigenic *S. pyogenes* infections may lead to streptococcal toxic shock syndrome, which can be life-threatening.***S. pyogenes*** can also cause disease in the form of postinfectious “nonpyogenic” (not associated with local bacterial multiplication and pus formation) syndromes. Theseautoimmune-mediated complications follow a small percentage of infections and include rheumatic fever and acute

postinfectious glomerulonephritis. Both conditions appear several weeks following the initial streptococcal infection. Rheumatic fever is characterised by inflammation of the joints and/or heart following an episode of streptococcal pharyngitis. Acute glomerulonephritis, inflammation of the renal glomerulus, can follow streptococcal pharyngitis or skin infection.

6. SUMMARY

The siddha system of medicine many formulations are available for the management of liver disease my selection of the drug *"**pavalaveerachunnam*** is a mineral, animal formulation taken from The Pharmacopoeia of siddha research medicines" authored by M. Shanmugavelu. *Chunnam* is the higher order dosage forms and specialized medicine in siddha system. *Siddhars* were the great scientist in ancient times. They used nano technology for the preparation of *chunnam.*Nano particles are easily absorbable, Biodegradable, Biocompatible of the human body.

Review of the literatures and lateral research works reveals that (coral and pavalam) ingredients of PVC used in treating liver diseases. In siddha system the liver disease is called ***kalleralnoi***. Liver disease is becoming ever more important. Deaths from liver disease are rising across the world.

Review of literature in various categories was carried out. Siddha aspect, Geological, zoological aspect and pharmaceutical review disclosed about the drug and the disease. Pharmacological review was done to establish the methodologies.

Review of literature from various siddha texts and modern science for the ingredients of the drug, which claims support as Hepato protective, Hypolipidemic, Diuretic and Lithotriptic activities.

The ingredients of the drug was identified and authenticated by the experts of *Gunapadam,* Geology department.

Since the trial drug ***pavalaveerachunnam*** was prepared according to the classical methods. The purification process of this drug possible to eliminates their toxins and increases its efficacy and the grinding, *Pudam* process of this drug helps to change the particle size of the drug for its better bio availability.

The ***pavalaveerachunnam*** was screened for various standardization parameters as per Siddha pharmacopoeial standards. The preclinical research out coming of the standardization can be used for evaluating the quality, purity and efficacy of ***pavalaveerachunnam***

According to the *thirithodam* theory the *pitha* increased in liver diseases. In panchabootha pasanam *veeram* is Appubootham. *Pavalam* is also a diuretic. *Pitham* is the Thee bootham so the line of treatment is called *Ehirurai.*

Physico-chemical analysis of *Pavalaveerachunnam* showed that loss on drying (LOD) is 0 % which shows that no moisture content present in the prepared medicine. Increased moisture content is the issue for instability of drug and lesser shelf-life of a drug. Since, *Pavalaveerachunnam* has been well prepared it could get maximum stability and better shelf-life. The total ash values of *Pavalaveerachunnam* were 3.9%, the value of total ash in the formulation is comparatively low. The value of total ash indicates that the inorganic contents of the formulation are below the limits. Acid-insoluble ash value of the prepared formulation (0.8%) shows that a very small amount of the inorganic component is insoluble in acid. It indicates that adulteration of raw ingredients by substances, such as silica and husk, is very less. The test drug *Pavalaveerachunnam* having, lower the acid insoluble value better will be the drug quality.43.3% of alcohol-soluble extractive value and 14.2% of water-soluble extractive value of the formulation shows that the mineral contents of the formulations are more soluble in alcohol than water and a lesser water soluble extractive value (14.2%) of the formulation. Hence honey should be used as adjuvant for this preparation. Specific pathogens like *Salmonella sp., Staphylococcus aureus, E.coli* and *Pseudomonas aeruginosa*are Nil. Hence, the test drug is free from any microbial contamination and it has standard quality.

Thin-layer chromatographyof*pavalaveerachunnam* shows under UV 254mm and 366mm. The RF value (0.93blue,0.46blue, 0.21blue, 0.14blue) indicates the presence of various inorgonic compounds in this drug.

From the result of preliminary **biochemical analysis** reveals that trial drug *pavalaveerachunnam* shows the presence of calcium, iron in ferric and ferrous form, sulphate, chloride, phosphate. These essential elements are necessary for the normal functioning of the body.

SEM analysis of the *Pavalaveerachunnam* shows most of the particles present in the sample is nano size, average particle size is 100-150 nano microns. So, very minimal quantity of the medicine is enough to treat the disease. *Siddhars* were the great scientist in ancient times. They used nano technology for the preparation of *Chunnam*. Nano particles have beneficial properties that can be used to improve drug delivery system. Nano particles are defined as particulate dispersion or solid particles with a size in the range of 1-100nm in diameter. They are easily Absorbable, Biodegradable, Biocompatible,

Non-antigenic in nature, Selective/Targeted/Controlled delivery of drugs to specific site of action in the body even across the blood brain barrier, Use to extend time window of bioavailability and to protect drug from enzymatic and chemical decomposition, Result in reduced peripheral side effect of drugs. The nano particles present in the drug results in a better bioavailability and facilitates absorption.

Fourier transform infrared spectroscopic studies of *Pavalaveerachunnam* shows the presence of the following functional groups at different peaks, they are Chloro alkanes, Vinyl - Trans - disubstituted alkanes, Aromatic - mono substituted benzene, potassium persulfate, copper, sulfate, carboxylic acid - acyl halides, Ammonium ions, Primary amines, Alcohols, Phenols.

Acute Toxicity study shows 2000 mg/kg dose of ***Pavalaveerachunnam*** did not produce any mortality and morbidity. Therefore the biological evaluation was carried out at a very conventionally safer range of 200 and 400mg/kg doses. In the present study showed even 2000mg/kg dose of VP did not produce any mortality and morbidity.

Therefore the biological evaluation was carried out at a very conventionally safer range of lOOmg, 200mg, 400mg/kg doses. The acute oral toxicity potentials of ***PavalaveeraChunnnam***in female Wistar albino rats were studied effectively. The acute toxicity result shows the test drug PVC does not produce any toxic signs and mortality up to its maximum dose level of 2000 mg/animal body weight in wister albino rats. Morphological characters like changes in skin, eyes, fur and nose appeared normal. It showed changes in touch response and decreased motor activity. The behaviorial changes are normal. According to OECD guidelines, for acute oral toxicity LD so dose up to 2000mg/ kg of the drug the test drug ***PavalaveeraChunnnam*** is a safe mineral animal drug and can be used for long time administration.

The average feed consumption was analyzed in group II and III animals in days (1,7,14,21 and 28) and compared with the group I control animals. The average food consumption increased significantly in all the groups from day 1 to 29 day.

The body weight of the different treatment groups were also estimated on the days (1,7,14,2land 28) to assess the toxicity of ***Pavalaveerachunnam***. The different treatment group animals shows significant increase in body weight starting from day 1 to day 29 was observed.

Sub acute Toxicity Study shows administration of ***Pavalaveerachunnam*** **in** different doses did not alter significantly the organ weights in group II and III. A mild increase in the weight of lung and kidneys was observed in Group III animals which were treated with 400mg / kg of *Pavalaveerachunnam*, which is reversible.

The hematological parameters (RBC, WBC, Platelets and Hb) were estimated in the different groups treated with *Pavalaveerachunnam*and were compared with the control animals (Group I). The WBCs increased significantly in Group III animals when compared with Group I. The Hb showed a significant increase in group III animals. Interestingly RBC count was significantly increased in Group III animals.

Pavalaveerachunnam administration in kidney function was also analyzed by estimating the levels of Glucose, Urea, creatinine and Protein the results are presented in table. Administration of ***Pavalaveerachunnam*** didnot alter the kidney function significantly.

The administration of ***Pavalaveerachunnam*** did alter the liver function.The total bilirubin levels didn't show any significant increase in all the groups when compared to the group I animals.

The effect of ***Pavalaveerachunnam*** in *the* biochemical parameters was analyzed by estimating the levels of key enzymes namely Cholesterol and Triglycerides and enzymes namely Albumin, Globulin, Total Bilirubin and Direct Bilirubin . The administration of ***Pavalaveerachunnam*** did alter the Albumin, Globulin, Total Bilirubin and Direct Bilirubin level.

Histopathology analysis shows heart, liver, kidney, spleen and all other organs are normal when compared with control.

Pharmacological analysis shows that the test drug *Pavalaveerachunnam* has got good significant. Hepatoprotective, Hypolipidemic, Diuretic, Lithnotriptic activity when compared to the standard drug. Thus it relieves the symptoms of liver disease.

From the above results, the test drug (PVC-50mcg) is resistant against ***Klebsiellapenumoniae, P.aeruginosa, E.coli, Salmonella typhi***and ***Candida albicans*** sensitive against ***Staphylococcus aureus,*** and ***Streptococcus pyogenus.*** Sensitive when compared to the standard drug (Amikacin-50mcg) and (Ketokonazole) which was evident from the zone of inhibition. As the above bacterial organisms responsible for pneumonia, bronchitis, pharyngitis, diarrhoea, typhoid fever, headache, urinary tract infection and skin

infectons. And fungal organism ***Candida albicans***responsible for superficial skin and mucosal infection. Our results confirmed the traditional use of PVC has antimicrobial activity.

Finally all the parameters and histopathological studies results revealed the drug was safe in rats. Acute oral toxicity of PVC observed no toxicity. Acute and sub acute toxicity study of *Pavalaveerachunnam* represents non toxic and safe drug in wistar albino rats, biochemical parameters and histopathology results it can be concluded that the dose level of PVC 200-400 mg mentioned in the Siddha Literature "*The Pharmacopeia of Siddha Research Medicine"* authored by Shunmugavelu M. is the safety dose for human consumption. So the test drug of PVC hope fully use for human trails.

7. CONCLUSION

The trial drug *PavalaveeraChunnam* is a mineral, animal formulation, selected from the text book of *THE PHARMACOPEIA OF SIDDHA RESEARCH MEDICINES* authored by Shumugavelu M. Dr. (Page no.86) for Hepatoprotective, Hypolipidemic, Diuretic, Lithotriptic activities and the results supported the study.

From the literature review, Physico-chemical analysis, Chemical analysis, Pharmacological studies, Anti Microbiology activities, Instrumental analysis and toxicological studies it is concluded that the test drug of PVC is safe and effective for ***Liver Diseases*** and in safer to continue even for a long duration.

8. FUTURE SCOPE

The trial drug ***pavalaveerachunnam*** has Hepato protective, Hypolipidemic, Diuretic and Lithotriptic activities in animal model which has been established in this study. However, the mechanism of action by ***pavalaveerachunnam*** which produced its effect on Hepato protective, Hypolipidemic, Diuretic and Lithotriptic activities in animal model need to be evaluated in a scientific manner and also multi-center clinical trials are also required to understand the exact molecular mechanisms of action. So it could be used worldwide in treatment of Liver diseases.

9. BIBLIOGRAPHY

1. Thiyagarajan .R, Gunapadam Thathu Jeeva vaguppu II and III,2nd edition publisbed by Indian Medicine and Homeopathy Chennai.
2. Shanmugavelu.M Dr The pharmacopoeia of Siddha research medicines Ist edition published by Sri G.D Naidu (Pvt Ltd Chunnam).
3. Ramachandran S.B Kosac Anupoga vaithya Bramha ragasiyam Ist edition published by Thamarai library chennai.
4. Sivan yagobhu chunna kandam 600 2nd edition published by Rathna printers -chennai.
5. Kuppusamy Muthaliyar B.S Anuboga vaithiya Bramha Ragasiyam 2nd edition published by Sri senbaga printers - Chennai.
6. Kannu Samy pillai .S Kannusamy paramparai vaithiyam 9th edition published by Thirumagal vijaya lashmi printers - Chennai.
7. Kuppusamy and uthamarayan .K.K Muthaliyar Dr K.N Siddha vaithiya thirattu 1st edition published by Indian Medicine and Homeopathy - chennai.
8. C.Kannusamy pillai C. Chikicharathna deepam 2nd edition published by Rathna nadar and sons chennai.
9. Hacheem.Pa .Muhammadha Apithulla sayaapu Anyboga vaithiya navaneetham 4th part 1 st edition 1995 Published by Thamarai library chennai.
10. K.Anbarasu ukimuni vadha kadam 1000 1st edition 1998 published by Thamarai library chennai.
11. S.B Ramachandran veeramunivar vagada thirattu 2nd edition 1994 published by Thamarai library chennai.
12. S.B Ramachandran sattai nigandu 1200 1st edition 1992 published by Thamarai library chennai.
13. Kuppusamy Mudaiyar K.N Utharmorayan C.S siddha vaidhya Thirattu,first Edition 1998 Department of Indian Medicine and Homeopathy chennai - 106.

14. Shanmughavelu.M Noigalukku siddha pariharram part 1.Third Edition 1999 Department of Indian Medicine of Homeopathy chennai -106.

15. Uthamorayan C.S Bhathathin siddha marunolugal First Edition 1984.Department of Indian Medicine and Homeopathy chennai-106.

16. Agathiyar vaidhya Rathina churukam second Edition 1998,Thamarai library chennai-26.

17. Narayana swami V.Pharmocopoeia of hospital of Indian medicine,second Edition 1995 ,Tamilnadu siddha medical board chennai -106

18. Kuppusamy mudaliyar K.N siddha maruthuvam (pothu) sixth edition 2004,Department of Indian medicine and homeopathy chennai-106.

19. Uthamaraayan C.S Siddha aruvai maruthuvam,fourth edition 2005,Department of Indian medicine and homeopathy chennai-106.

20. Pathiren siddhars halvaidhya sillarai kovai(part 2) Second edition - 2005,Thamarai library -7 N.G.O Colony vadapalani chennai-26.

21. Shanmugavelu.M Noigalukku siddha parigaram -II Part,Third edition 1999 Department of indian medicine and homeopathy chennai -106.

22. Thiyagarajan.R siddha maruthuvam (sirappu) second Edition 1995 Department of Indian medicine and homeopathy chennai -106

23. Utharamaraayan.C.S Tholrakriama Araichiyum siddha maruthuva varalaarum First edition 1978 Department of Indian medicine and homeopathy chennai - 106.

24. Davidson's principles and practice of medicine 10th edition 2002 Elsevier science Ltd.

25. Sambasivampillai T.V Tamil to English Dictionary,2rd edition 1998 Department of Indian medicine chennai -106.

26. Murugesa mudaliyar .K.S Nanju mauivu nool 4th edition 2005 Department of Indian Medicine and Homeopathy Chennai -106.

27. Pon.Guruechiromani,Balavagadam,3rd edition 1992 Department of Indian medincine and homeopathy chennai 106.

28. Thirunarayan.T An Introduction to siddha medicine 1st edition,Thirukumaran publish ers Thirucendur.

29. Siddha Toxicology A translated text of nanju murivunool 1st edition 1999 Department of Indian medicine and homeopathy chennai-106.

30 A.B Nybakken James 1997 Marine Biology ,4th edition Menlopark CA Addition wesley.

31. Achituv .Y and Dubinsky .Z 1990 Evolution and Zoogeogaraphy of coral reefs Eco systems of the world volume 25.

32. Allision .N(1996) Geochemical anomalies in coral skeletons and their possible implications for paleo envirnomental analysis mari chem volume 55.

33. Aruthur.R (1996) "A Survey of the coral reef of the mahatma gandhi marine national park" A report submitted to ANET.

34. Birkeland .C (1997) life and death of coral reefs International Thomson Publishing,Newyork.

35. EDWARD SALIS BURY DANA.A Textbook of MINERALOGY Fourth edition published by CBC Publishers,11 Darya Ganj,New Delhi -110002.

36. Dana's Textbook of Mineralogy 10th edition 1986 published by The World press pvt Ltd.32A,College street Calcutta.

WEB SITE

www.coral film.com

www.coral reef.org

www.reefbase.org

www.reefimage.com

www.seaworld.org

www.sn2000taxonomy.in

www.oceanworld.edu

www.uvi.edu/coralreefer